Molecular Analysis of the Translocation Breakpoint in a Philadelphia-positive, bcr-negative ALL Patient*

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A. Introduction

Translocation t(9; 22) (q34; q11) occurs in 90% of patients with chronic myeloid leukaemia (CML) [13] and in 5% of children and 10%–20% of adults with acute lymphoblastic leukaemia (ALL) [16]. It is easily identified by the $22q^-$, or Philadelphia, chromosome. In CML all chromosome 22 breakpoints are located within the 5.8-kb breakpoint cluster region (bcr) in the 3′ part of the *phl* gene [6]. In ALL, however, only some breakpoints are in bcr. Others are more 5' in the *phl* gene, as suggested by observations made at the RNA and protein level [3, 4, 9, 17] and at the DNA level by pulsed-field gel electrophoresis [14]. Both in CML and in ALL the breakpoint on chromosome 9 is within the *abl* oncogene upstream of the common exon [7].

Depending on the breakpoint on chromosome 22, a hybrid 8.5-kb mRNA [15] and a 210-kDa fusion protein [10] are formed (in Ph^+ bcr^+ CML and ALL), or a hybrid 7-kb mRNA and a 190-kDa fusion protein (in Ph^+ bcr^- ALL) [1–4, 9, 11, 17]. Both protein products seem to have an enhanced tyrosine kinase activity compared with the 145-kDa *abl* protein when assayed in vitro.

In order to characterize a p190 ALL breakpoint at the genomic level we have cloned and sequenced the breakpoint of the $9q^+$ chromosome from a Philadelphia-positive p190 ALL patient.

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B. Results and Discussion

We have shown previously that a c-*abl*-related 190-kDa protein is expressed by the leukaemic cells of patient F. Y. investigated here [1]. When DNA from the leukaemic cells was analyzed by Southern blotting and hybridization with a probe spanning the bcr region, no rearrangements were found. After hybridization with a c-*abl* probe, however, a rearranged band was detected in *Bgl*II and *Bam*HI digested DNA from the leukaemic cells which was not present in the DNA from a skin biopsy of the same patient [1, 5]. Using the same c-*abl* probe (0.52 E in Fig. 1 a) the rearranged 13.4-kb *Bgl*II fragment was isolated from an EMBL3 library made from DNA from the patient's leukaemic cells [5]. The 11.2-kb *Bgl*II fragment spanning the corresponding germline chromosome 9 region was isolated from the same library. Comparison of the restriction maps of the two *Bgl*II fragments showed we had cloned the $9q^+$ junction fragment (Fig. 1 b). As in CML, the breakpoint on chromosome 9 is upstream of the common exon a2, here between exon Ia and a2. Probes FY1 and FY2 from the non-chromosome 9 part of the clone (Fig. 1 b) were used to hybridize to Southern blots of DNA from several rodent-human somatic cell hybrids. In this way we found that the non-chromosome 9 sequences are derived from chromosome 22. Comparison with a genomic clone of the *phl* gene (Fig. 1 d, λbcr 5.7) which contains the second exon and 11.7 kb of the 3′ portion of the adjacent intron, revealed that the cloned $9q^+$ junction fragment contains part of this *phl* intron. There-

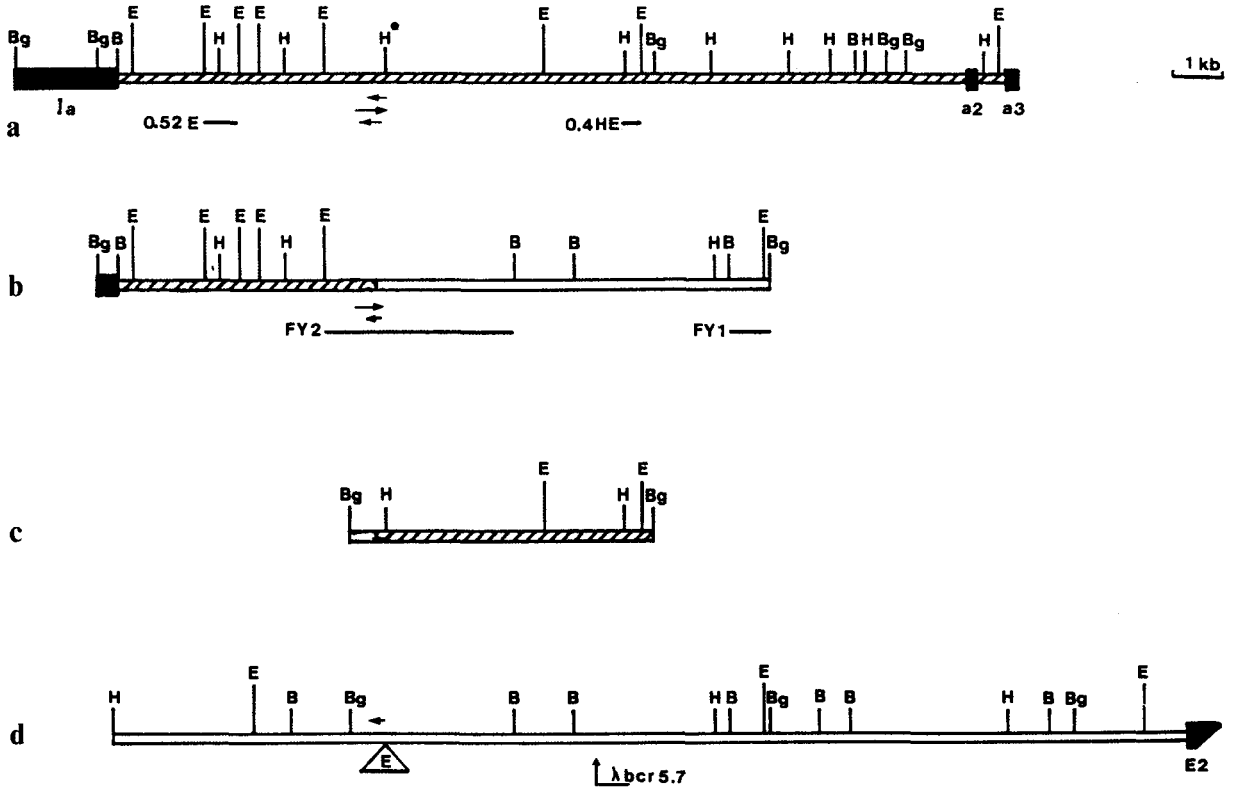

Fig. 1a–d. Restriction enzyme maps of the junction regions of the DNA of the Ph-positive p190 ALL compared with the normal regions of chromosomes 9 and 22. Abbreviations of restriction enzymes: *B, Bam*HI; *Bg, Bgl*II; *E, Eco*RI; *H, Hind*III. The *Hind*III site in (**a**) which is marked by an asterisk is described in the text. Horizontal arrows in (**a, b, d**) denote the sequencing strategy. *Hatched* and *white regions* represent chromosome 9 and chromosome 22 respectively. **a.** Germline c-*abl*, adapted from [7]. Probes 0.52 E and 0.4 HE are indicated below the map. Exons are indicated by *black boxes*. **b.** The cloned *Bgl*II fragment from the $9q^+$ chromosome. Probes FY1 and FY2 are shown below the map. **c.** The size of the chromosome $22q^-$ *Bgl*II fragment is derived from the $9q^+$ map in comparison with the germline c-*abl* and *phl* restriction enzyme maps. **d.** The *phl* map is compiled from a genomic clone [9], the $9q^+$ junction fragment and data from Southern blots of DNA from several patients after hybridization to probes FY1 and FY2. The exact size of *phl* exon 2 (E2) is not known; its location has been determined by hybridization with a cDNA probe. The *arrow* indicates the position of the 5′ end of the λbcr 5.7 genomic clone. The *triangle* below the map denotes the approximate location of a 1-kb deletion polymorphism containing an *Eco*RI site. Additional *Bam*HI and *Bgl*II sites may exist

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                    10        20        30        40        50        60        70        80        90       100
chrom. 9    ACAAAAATCTCATTTCTTTTTTTTTTTGAGACAAGAGTCTCACTCTGTCGCCCAGACTGGAGTGCAGTTGCACGATCTCAGCTCACTGCAAGCTCCGCCT
chrom. 9q+  ACAAAAATCTCATTTCTTTTTTTTTTTGAGACAAGAGTCTCACTCTGTCGCCCAGACTGGAGTGCAGTTGCACGATCTCAGCTCACTGCAAGCTCCGCCT
chrom. 22   TCAGATTTTTTCAAGGAGGGGTGTCTTAGTCACTTCAGGCTGCTATAACAAAAATGCCATAAACTGGGTACCTTAAACAACAAACACTTACTCCTCACAG

                    ↓ 110     120       130       140       150       160       170       180       190       200
chrom. 9    CCTGGGTTCACGCCATTCTCCTGCCTCAGCCTCCCTAGTAGAGGGTACTACAGGCGCCCGGGGGTTTCACCATGTTAGCCAGGATGGTCTCCATCTCCTG
chrom. 9q+  CCCTGGAGGCAGGAAGTCAGTATCAAGGAGCTGGAACATTTGGAGTCTGCCAAGGGCCCACTTCCTGGTTCTTAGCCATCTTCTTGCTGTGTTCTCACCT
chrom. 22   TGCTGGAGGCAGGAAGTCAGTATCAAGGAGCTGGAACATTTGGAGTCTGCCAAGGGCCCACTTCCTGGTTCTTAGCCATCTTCTTGCTGTGTTCTCACCT
                    ↑

                    210       220       230       240       250       260       270
chrom. 9    ACCTCGTGATCCGCCCACCTCGGCCTCCCAAAGCCCTGGGATTACAGGCGTGAGCCACTGCACCGGGCCA
chrom. 9q+  GGTGTGGAAAGGATGGGGGCTGTCTCTGGGGTTTCAAAAGGGCACTAATCCCCTTCATGAGGGTTCCGTC
chrom. 22   GGTGTGGAAAGGAT
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Fig. 2. Nucleotide sequence of the chromosome 9, $9q^+$, and 22 regions around the breakpoint. Normal chromosome 9, and the $9q^+$ sequences are from patient F.Y.; chromosome 22 sequences are from non-ALL DNA. The breakpoint is indicated by arrows between 102 and 103. Sequences showing homology to Alu sequences are underlined. Between 160 and 161 there is a 40-bp stretch of Alu sequences missing. Numbers are arbitrary. The *Hind*III site marked with an asterisk in Fig. 1a is not shown here; it would be at number 368

fore, we conclude that the breakpoint on chromosome 22 in this patient is located in the putative first intron of the *phl* gene, approximately 16.5 kb upstream from exon 2. This location is in agreement with results from the analysis of the RNA and the protein which result from this type of translocation [3, 4, 9, 17]. The chromosome 22 breakpoint in the ALL cell line SUP-B13 [14] lies within the same *Bam*HI fragment as the breakpoint in patient F.Y. described here. Using probes FY1 and FY2 (Fig. 1 b) we detected rearrangements in the DNA from two of 12 additional Ph-positive p190 ALL patients.

We determined the nucleotide sequence around the breakpoint from double-stranded templates using the Sequenase kit (United States Biochemical Corporation). Oligonucleotide primers were prepared on an Applied Biosystems 381A DNA synthesizer. The sequencing strategy is indicated in Fig. 1 a, b and d; part of the sequence is shown in Fig. 2.

The chromosome $22q^-$ junction fragment has not been cloned; its restriction map in Fig. 1 c has been predicted from the chromosome 9, $9q^+$, and 22 maps. When c-*abl* probe 0.4 HE (indicated in Fig. 1 a) was hybridized to a Southern blot of *Bgl*II-digested DNA from the patient's leukaemic cells, a rearranged band of 6.2 kb (the predicted size in Fig. 1 c) was observed in addition to the expected germline 11.2-kb fragment [5]. The nucleotide sequence of the chromosome $22q^-$ junction fragment will be obtained after amplification from the patient's DNA by the polymerase chain reaction. So far, there is no evidence for large insertions or deletions at this breakpoint. The breakpoint on chromosome 9 is 265 bp 5′ of the *Hind*III site, which has been marked with an asterisk in Fig. 1 a. There is 88% homology between the c-*abl* sequences around the breakpoint and a consensus Alu repeat sequence, when a 40-bp stretch of Alu which is missing between nucleotide 160 and 161 is omitted. Homology to Alu sequences is occasionally observed near translocation breakpoints, e.g., in the CML t (9; 22) translocation [8, 12]. In chromosome 22, however, there is no Alu homology in the region described here (Fig. 2), which makes recombination between Alu sequences as a mechanism for translocation unlikely.

C. Conclusions

We have shown by cloning of the genomic DNA from the leukaemic cells of a Ph^+ p190 ALL patient that the translocation breakpoint on chromosome 22 is located in the first putative intron of the *phl* gene. It is clear that in Ph^+ p190 ALL the chromosome 22 breakpoints are not located in such a small region that they can be detected with one probe, as is the case in Ph^+ bcr^+ CML. The nucleotide sequence around the breakpoint described here does not reveal any obvious indication for the mechanism of the translocation.

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Note added in proof:

More extended sequence data and the exact restriction map around the breakpoint have been described in: van der Feltz et al. (1989) Nucl Acids Res 17:1–10.