Long-Range Mapping of the Philadelphia Chromosome by Pulsed-Field Gel Electrophoresis

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The Philadelphia chromosome (Ph^1) of chronic myelogenous leukemia (CML) contains sequences from chromosome 9, including the ABL protooncogene, that have been translocated to the breakpoint cluster region (bcr) of chromosome 22, giving rise to a bcr-ABL fusion gene, whose product has been implicated in the genesis of CML. Although chromosome 22 translocation breakpoints in CML virtually always occur within the 5.8-kilobase (kb) bcr, chromosome 9 breakpoints have been identified within the known limits of ABL in only a few instances. For a better understanding of the variability of the breakpoints on chromosome 9, we studied the CML cell line BV173. Using pulsed-field gel electrophoresis (PFGE), large-scale maps of the t(9;22) junctions were constructed. The chromosome 9 breakpoint was shown to have occurred within an ABL intron, 160 kb upstream of the v-abl homologous sequences, but still 35 kb downstream of the 5'-most ABL exon. bcr-ABL and ABL-bcr fusion genes were demonstrated on the Ph^1 and the 9q+ chromosomes, respectively; both of these genes are expressed. These results suggest that the 9:22 translocation breakpoints in CML consistently occur within the limits of the large ABL gene. RNA splicing, sometimes of very large regions, appears to compensate for the variability in breakpoint location. These studies show that PFGE is a powerful new tool for the analysis of chromosomal translocations in human malignancies.

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MATERIALS AND METHODS

Cell lines. BV173 was established from the malignant cells of a patient with CML in blast crisis.4 Other lymphoid cell lines used as controls are Molt 16,11 RCH-ACV,13 and SUP-B13.15

Cytogenetic analysis. Cytogenetic analysis with a trypsin-Giemsa banding technique was done on metaphase cells of the BV173 cell line in logarithmic phase growth. Chromosome abnormalities are described according to the International System for Human Cytogenetic Nomenclature.

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Southern hybridization and DNA probes. Southern blots were prepared according to standard methods and hybridized under high-stringency conditions. DNA probes were labeled with 32P by the random-primer method of Feinberg and Vogelstein. The following ABL probes were used for these studies: pHAb15', a 0.85-kb BamHI/HindIII fragment containing sequences of exon Ib; T39-1, a 1.0-kb EcoRI fragment containing chromosome 9 sequences immediately 3' to the translocation breakpoint in the CML cell line K562; T28-2, a 0.6-kb BamHI fragment from an intron in the v-abl homologous region; and ABL-SS, a 0.8-kb SstI fragment cloned from a t(9;22) breakpoint in a patient with CML. The bcr exon 4 probe is a 1.3-kb HindIII/EcoRI genomic probe containing sequences homologous to this exon. Inversion Cytogenetic studies at the time of our molecular analysis demonstrated the typical 9q+ chromosome and three copies of the Ph1. No normal homologue of chromosome 9 was present; instead, there were two copies of a derivative of chromosome 9, which resulted from a translocation between the long arms of chromosomes 8 and 9, and involved bands q11 and q13. Because the reciprocal product of the t(8;9) was not present, the effect of this translocation was a loss of chromosome 9 material distal to band q13, including the site of the normal ABL locus at band q34. The complete karyotype of BV173 was 48, X, –Y, –9, der(1)(pter→q42::1q32→1q42::1qter), del(6)(q13q21), i(8q), inv(12) (p13q13), +der(9)(t8;9) (q11q13), +der(9)(t8;9) (q11q13), t(9;22)(q34;q11), +der(22)t(9;22)(q34q11), +der(22)t(9;22)(q34q11).

Identification of the translocation breakpoint within bcr. Hybridization of the 3'-bcr probe to BglII and BamHI digests revealed germline (5.0 and 3.3 kb, respectively) and rearranged (9.5 and 6.6 kb, respectively) bands in each, whereas only a germline band was identified in a HindIII digest. Based on a published restriction map of bcr, these data indicated that chromosome 22 breakpoint in this cell line was ~1.0 kb 5' of the sequences recognized by the probe (Fig 1B). Because sequences distal (3') to the breakpoint are translocated to chromosome 9, restriction fragments that hybridize to the 3'-bcr probe must originate from either the 9q+ or the normal chromosome 22.

Analysis of the 9q+ chromosome. Probes from the 5' and 3' sides of the breakpoint within bcr (Fig 1B) were used to identify restriction fragments originating from the Ph1 and the 9q+ chromosomes, respectively. DNA from BV173 was digested with the restriction enzymes NotI and SFI, and fractionated by orthogonal-field alternative gel electrophoresis. Hybridization of NotI digests to the 3'-bcr probe revealed a 350-kb rearranged band that also hybridized to the pHAb15' and T39-1-2 probes (Fig 2A), which are derived from the extreme 5' end of ABL. Similarly, in SFI digests, a rearranged band of ~35 kb hybridized to both 3'-ber and T39-1-2 probes (Fig 2B). Therefore, these rearranged bands contain the translocation junction of the 9q+ chromosome. As expected, neither of these rearranged bands hybridized to probes from within the ABL common exons (not shown), which had been translocated to the Ph1. The location of the chromosome 9 breakpoint was determined by reference to a restriction map of bcr that includes SFI sites. Because SFI sites occur 3 kb 3' and 3 kb 5' of the bcr breakpoint, the translocation breakpoint on chromosome 9 in BV173 lies just <35 kb downstream of exon 1b, or 160 kb upstream of exon II, within the ABL first intron. Thus, exon 1b is separated from all other known coding regions of ABL (Fig 1C).

Analysis of the 22q– chromosome. The Ph1 (22q–) chromosome was mapped by hybridization of NotI digests of BV173 DNA to probes from the 3' end of ABL, the 3' and 5' sides of bcr, and IGLC. The 3' ABL probe (T28-2-2) recognizes sequences distal to the translocation breakpoint on chromosome 9, and the 5'-bcr probe recognizes sequences proximal to the translocation breakpoint on chromosome 22; therefore, each is expected to hybridize to DNA from the 22q-chromosome. The Hu-λ-C2 also was used for this study because IGLC lies proximal to bcr on the q-arm of chromosome 22, (Fig 1B) and invariably remains on the Ph1 chromosome in CML. Field-inversion gel electrophoresis was used to obtain better resolution of the extremely high mol wt bands produced by NotI. As shown in Fig 3, the
Hu-\(\lambda\)-C\(_2\) probe hybridized to two \(\text{Notl}\) bands of high mol wt in the lane containing BV173 DNA, but to only a single band within the same size range in DNA from a cell line that did not have a t(9;22). The variation in mobility of the germline bands which hybridize to Hu-\(\lambda\)-C\(_2\) is an artifact of inversion-field electrophoresis, and is due to variations in the amount of DNA in a lane; the germline band in BV173 appears to be the same size as that seen in at least six other cell lines when examined by other gel systems (C.A. Westbrook and C.M. Rubin, unpublished observations). These bands are at \(-1,200\) and \(1,600\) kb, as determined by the relative migration of yeast chromosomes of known size.\(^3\) Both the 3'-\(bcr\) and the 5'-\(bcr\) probes, but not the \(\text{ABL}\) probe T28-2-2, hybridized to the single band in the control and to the larger of the BV173 bands, identifying these as germline bands. The faster migrating band in BV173, however, hybridized to Hu-\(\lambda\)-C\(_2\), 5'-\(bcr\), and T28-2-2, but not to 3'-\(bcr\), demonstrat-

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**Fig 1.** Long-range restriction maps of the normal \(\text{ABL}\) gene, the normal \(bcr\) region, and the translocation junctions of BV173. Chromosome 9 sequences are depicted by thin lines, and chromosome 22 sequences by thick lines. (A) PFGE map of the \(\text{ABL}\) gene. Known \(\text{ABL}\) exons are shown as dark boxes on the line; the two alternative first exons are labeled Ia and Ib; exon II is the common second exon for both exons Ia and Ib. Sequences homologous to \(\text{v-abl}\) include all of the exons that are 3' to exon II. Arrows indicate the approximate locations of the human genomic \(\text{ABL}\) probes. \(\text{Notl}\) and \(\text{SfiI}\) restriction fragment sizes are given in kilobases. (B) PFGE map of the \(bcr\) region on chromosome 22. Neither the full extent of the \(bcr\) gene nor its distance from \(\text{IGLC}\) has been determined. Inset shows immediate vicinity of the breakpoint cluster region, location of genomic probes used in this study, and region of the BV173 translocation breakpoint. Restriction sites diagrammed are B, \(B_{am}\), Bg, \(B_{gi}\), E, \(E_{co}\), H, \(H_{ind}\), I, \(S_{fi}\). (C) Structure of 9q+ chromosome at translocation junction. Location of the probes used to determine this structure and approximate location of \(\text{Notl}\) and \(\text{SfiI}\) restriction fragments are shown. (D) Structure of the 22q− chromosome at translocation junction, showing location of \(\text{IGLC}\), 5'-\(bcr\), and \(\text{ABL}\) probes used to determine the structure, as well as the \(\text{Notl}\) and \(\text{SfiI}\) restriction fragments.

**Fig 2.** PFGE analysis of the 9q+ chromosome in BV173. \(\text{Notl}\)(A) and \(\text{SfiI}\) (B)-digested DNA was fractionated by orthogonal-field alternation gel electrophoresis and sequentially hybridized to \(bcr\) and \(\text{ABL}\) probes. (A) Lanes 1 and 2 contain DNA from cell lines \(\text{RCH-ACV}\) and BV173, respectively. (B) Lanes 1, 2, and 3 contain DNA from cell lines \(\text{RCH-ACV}\), \(\text{SUP-Bi}\), and BV173, respectively. Panels are labeled according to specific probe used. Mol wts of the hybridizing fragments, estimated from the relative migration distances of bacteriophage \(\lambda\) multimers, are given in kilobases to the left of each autoradiogram. Germline and rearranged bands are indicated with lines and arrows, respectively.
Phoreresis and sequentially hybridized to BV173 (lanes 2). fractionated by field-inversion gel electrophoresis and sequentially hybridized to IGLC, bcr, and ABL probes. Panels are labeled according to specific probe used. Mol wts of the hybridizing fragments, estimated from the relative migration distances of bacteriophage λ multimers and intact yeast chromosomes, are given in kilobases to the left of each autoradiogram. Arrowheads indicate positions of two yeast chromosomes of known molecular size. Germline and rearranged bands are indicated with lines or arrows, respectively.

Fig 3. PFGE analysis of the 22q - (Ph') chromosome in BV173. NotI-digested DNA from the lymphoid cell line RCH-ACV (lanes 1) and BV173 (lanes 2), fractionated by field-inversion gel electrophoresis and sequentially hybridized to IGLC, bcr, and ABL probes. Analysis was facilitated by loss of the normal ABL allele in BV173, which had been suggested by cytogenetic studies and was confirmed by the absence of germline ABL fragments on PFGE analysis (Figs 2 and 3). Thus, all ABL transcription in BV173 occurs from either the 9q+ or the Ph' chromosome. Transcription of a complete 7.0-kb message containing exon lb is impossible since the normal ABL locus is absent and since exon lb cannot be spliced to the common exons, which have been translocated. On the other hand, the type la transcriptional unit is intact on the Ph' chromosome, therefore, complete 6.0-kb type lb mRNA can be produced if a functional promoter is present.

Northern blots were prepared from poly-A–selected RNA of BV173 cells and from the lymphoid cell line RCH-ACV used as a control. Hybridization to the ABL cDNA probe containing portions of the ABL common exons revealed the expected 6.0-kb type la and 7.0-kb type lb ABL messages in the control cell line. In BV173, the 6.0-kb type lb transcript and the characteristic 8.5-kb bcr-ABL transcript were seen (Fig 5). Although the amount of the 6.0-kb transcript in BV173 cells appears to be roughly comparable to that seen in the control cell line, it is much less abundant than the larger, 8.5-kb message that originates upstream. Our previously reported immunoprecipitation analysis of BV173 confirmed that these cells produced an ABL p145 protein of normal size and abundance, which presumably originates from this 6.0-kb mRNA.

Rehybridization of the Northern blot to the exon lb probe pHab15' confirmed that the normal 7.0-kb ABL message was
the same biologic effect, despite a heterogeneity of the breakpoint location in ABL.

These results also lead to several interesting conclusions about the structure and function of ABL on the normal chromosome 9 and the Ph' chromosome. First, it is clear that transcription of ABL message can initiate independently at exon Ia and at exon Ib and does not require an intact ABL locus. Second, it is apparent that a normal type Ib transcript is not essential for proliferation of BV173 cells. Third, the Ph' translocation leaves the type Ia transcriptional unit intact in most cases of CML, since most breakpoints occur upstream of exon Ia. Evidence also suggests that methylation of the type Ia promoter can occur following its translocation to the Ph' chromosome, possibly leading to modulation of expression of this message. One could speculate that type Ia transcription on the Ph' plays a role in the disease process if, for example, it interferes with transcription of the bcr-ABL fusion message or if the normal ABL protein competes with the p210bcr-ABL protein for binding sites.

The existence of a transcriptionally active 5' ABL-3' bcr fusion gene on the 9q+ chromosome may be a frequent occurrence in CML. Whether this gene produces a protein product or has any other biologic function is not known; however, it is unlikely to play a major role in the pathogenesis of CML, since events that occur on the 9q+ chromosome are not observed consistently in all patients with the t(9;22). That is because ~8% of patients with CML have complex variant Ph' translocations, in which the chromosome 9 derivative contains sequences from a chromosome other than 22 and therefore cannot contain a 5' ABL-3' bcr fusion gene such as that found in BV173.

In this study, we showed that PFGE is useful for analysis of human chromosomal translocations. Rearrangements within the 230-kb ABL gene were easily demonstrated with this method, whereas conventional Southern blot analysis of a region of this size would have been virtually impossible. PFGE will undoubtedly prove useful for the analysis of other neoplasms that are characterized by specific chromosomal rearrangements, including those postulated to involve the ABL gene, such as Ph'-positive acute lymphoblastic leukemia and rare cases of Ph'-negative CML.

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