Frequent and Extensive Deletion During the 9;22 Translocation in CML

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Chromosomal translocation is one mechanism by which cellular oncogenes may be activated during tumorigenesis. The translocation of the abl oncogene to the Philadelphia chromosome in chronic myelogenous leukemia (CML) results in a new RNA transcript that fuses sequence from chromosome 22 to sequence from the abl oncogene. This RNA presumably codes for a new abl-related protein product found in CML, the activity of which is different from the normal abl protein. The molecular structure of the translocation varies from patient to patient, and the individual variation in RNA transcript and protein product remains to be defined. This report describes the frequent occurrence of chromosomal deletion within the 9q+ chromosome during these translocations. The location of the deletions suggests that some mechanism maintains the chromosomal breakpoint on the Philadelphia chromosome within a limited region. These deletions complicate the interpretation of Southern blots as a means of detecting the translocation.

The Philadelphia (Ph) chromosome in chronic myelogenous leukemia (CML) was the first human chromosomal abnormality consistently associated with a malignancy.1,2 The 9;22 translocation which creates the Ph chromosome moves the abl oncogene from chromosome 9 to chromosome 22. The extent of the normal cellular abl (c-abl) gene has not yet been defined, but the regions on chromosome 9 which are homologous to the transforming gene of the Abelson murine leukemia virus (v-abl) have been identified.3,4 The breakpoint on chromosome 9 is sometimes found 4 to 6 kilobases (kb) 5' to this v-abl homologous region, but is most often much further 5'.5-7 The minimum amount of abl sequence from chromosome 9 which must move to chromosome 22 to generate a productive Ph chromosome is not yet known. In the cases thus far examined, the portions of c-abl that are homologous to the viral gene always translocate to the Ph chromosome. In contrast to the wide variation in the chromosome 9 breakpoint location, reports to date have suggested that the breakpoints on chromosome 22 occur within a region of approximately 6 kb, the breakpoint cluster region (bcr).7

The normal abl gene produces two RNA transcripts approximately 6 and 7 kb in size, and a protein with a molecular weight of 145 kilodaltons (kd).8,9 After the abl gene is translocated to the Ph chromosome, a new 9 kb abl-related RNA species is produced.10-12 This new RNA begins in the chromosome 22 sequence and continues across the translocation junction into the abl sequence.13,14 This newly created fusion gene of chromosome 22 and chromosome 9-abl sequence presumably codes for a new protein found in CML with a molecular weight of 210 kd.15-17 The protein kinase activity of this new protein has been reported to differ from that of the normal abl protein.18

The four chromosomes present before and after the translocation are diagrammed in Fig 1. It is possible to detect the breakage and reunion of the chromosomes by using DNA fragments from either chromosome 9 or chromosome 22 as probes to detect novel restriction fragments that span the translocation junction point.

The present study employed two probes from the bcr to localize the chromosome 22 breakpoints in CML patients. In this analysis, we demonstrate that deletion of chromosome 22 sequence occurs frequently during this translocation. However, even in the presence of these deletions, the 3' end of the chromosome 22 portion of the Ph chromosome always terminates within the bcr, suggesting that there is some vital sequence located near the 5' end of the bcr. In addition, the frequent occurrence of deletion in this area means that 3' bcr probes will not identify the rearrangement in certain patients. To consistently identify the 9;22 translocation will at least require the use of a probe from the 5' portion of the bcr.

MATERIALS AND METHODS

DNA was isolated from the peripheral white blood cells of CML patients using methods that have previously been described.1 All the patients described in this study had a karyotype determined, and all patients had both Ph and 9q+ chromosomes. Some patients had additional chromosomal abnormalities. It was essential to obtain a

Fig 1. Schematic representation of the 9;22 translocation products and the production of the Ph chromosome. (A) Normal chromosome 9. The dashed line indicates the chromosomal breakpoint. The v-abl homologous region of the abl oncogene is indicated on chromosome 9. The breakpoint location on chromosome 9 is variable; in this example it is located 5' to the region homologous to the v-abl gene. (B) Normal chromosome 22. The breakpoint location on chromosome 22 is within the bcr. (C) The Ph chromosome. The 5' portion of the Ph chromosome comes from chromosome 22, the 3' portion comes from chromosome 9. (D) Generation of the 9q+ chromosome. The 5' portion of the 9q+ chromosome is from chromosome 9, the 3' portion from chromosome 22.
fragment of chromosome 22 to analyze the patients' breakpoints.
Restriction mapping of the patient FP, described in an earlier
publication, had shown that the 9q+ chromosome translocation
junction point was contained within a 5.0 kb Hind III fragment (Fig
3). A recombinant DNA library was constructed in the phage vector
charon 21a, and approximately $1 \times 10^6$ phage were screened using a
Hind III-Eco RI fragment of chromosome 9 located just 5' of the
v-abl homologous region. The 5 kb Hind III fragment containing
the translocation junction was isolated and subcloned into the
bacteriophage pSP64 (Promega Biotech, Madison, Wis). The Bgl II
fragment of this clone (probe 2 in Fig 2) was then used to screen a
normal human recombinant library. A clone containing the normal
4.2 kb Hind III fragment of chromosome 22 was isolated, and the 1.2
kb Hind III-Eco RI fragment (labelled probe 1 in Fig 2) was
subcloned from it. These two probes were used to examine Southern
blots of CML patients. These blots generated the data shown in Figs
4 and 5, and Tables 1 and 2.

### RESULTS

Using a v-abl fragment as a probe to screen a normal
human recombinant library permitted the isolation of human
c-abl fragments which were then employed as probes to examine Southern blots of CML patients. These probes identified two patients whose chromosome 9 breakpoints were within 4 to 6 kb of the v-abl homologous region. The restriction analysis of the 9q+ chromosome from one of these patients, TH, is summarized in Fig 3. The restriction map of the 9q+ chromosome from TH provides a linear map of the restriction enzyme sites located in chromosome 22 sequence immediately 3’ to the 9q22 junction. By aligning these restriction sites with the known restriction map of chromosome 22 (Fig 3), these data suggest that the chromosome 22 breakpoint in the 9q+ chromosome is not within the bcr in patient TH.

To further localize the chromosome 22 breakpoint in TH, and to identify the chromosome 22 breakpoints in other CML patients, cloned probes from chromosome 22 were used. The probes labelled 1 and 2 in Fig 2 were used to examine Southern blots of 14 CML patients and 1 CML cell line (Table 1). Representative lanes from these Southern blots are shown in Figs 4 and 5. The more 3’ chromosome 22 probe, #2 in Figure 2C, identified breakpoints within the bcr in 6 of our 14 patients. The abnormal restriction bands found in the two CML patients and the CML cell line shown in Fig 4 indicate that a breakpoint within the bcr is present. This is consistent with previously reported data.

The individual whose Southern blots are shown in Fig 5A is the patient TH, whose 9q+ chromosome digests were summarized in Fig 3, and who represents a group of 4 patients who were initially perplexing. All these patients had Ph and 9q+ chromosomes visualized in a karyotype. The chromosome 22 probe labelled #1 in Fig 2 identified not only the normal but also an additional abnormal restriction band (Fig 5A, lanes 3 to 5). These bands are drawn schematically in Fig 6. This indicated that the patient had a fragment of chromosome 22 that ended within the bcr joined to a fragment of chromosome 9. Figure 5A, lane 3, shows the abnormal 7.1 kb Bgl II band; lane 4, the abnormal 13 kb Kpn I band; and lane 5, the abnormal 4.4 kb Bam HI band which are shown in Fig 6 aligned with the map of chromosome 22. These abnormal restriction bands make it possible to define the end of the chromosome 22 fragment that becomes part of the Ph chromosome. As shown in Fig 6, chromosome 22 sequence on the Ph chromosome ends within the bcr for patient TH. We will call the end of chromosome 22 which joins the Ph chromosome the Ph chromosome/chromosome 22 breakpoint (Ph-22-bkpt). This data from chromosome 22 probes contradicted the earlier data summarized in Fig 2 that indicated that the chromosome 22 breakpoint on the 9q+ chromosome was located at least 8 kb 3’ to the bcr. The simplest explanation for these data is that the chromosome 22 sequence lying between the Ph-22-bkpt and the 9q+ chromosome/chromosome 22 breakpoint (9q+ -22-bkpt) had been deleted during the translocation. This is illustrated in Fig 7.

![Southern blots of a single patient, TH, representing the group of patients who showed only normal bands with the more 3'-chromosome 22 probe, but were found to have breakpoints within the bcr when probed with the more 5'-chromosome 22 probe. Arrowhead indicates normal band; asterisk indicates abnormal band. The lanes are from different gels, so fragment size and distance migrated are not comparable from one lane to another. Lanes 1–2: Probe 2, the 3’ probe. Lanes 3–5: Probe 1, the 5’ probe. Lane 1: Xba I digest with normal 9.4 kb band. Lane 2: Eco RI digest with a normal 16.7 kb band. Lane 3: Bgl II digest, normal 4.9 kb band, abnormal 7.1 kb band. Lane 4: Kpn I digest, normal 20 kb band, abnormal 13 kb band. Lane 5: Bam HI digest, normal 3.2 kb band, abnormal 4.4 kb band. The 3 abnormal bands are illustrated in Fig 6.](A)

![Southern blots of a second patient, ES, who also had a bcr breakpoint when probed with probe 1, but showed only normal bands when probed with probe 2. Lane 1: probe 2, Eco RI, normal 16.7 kb band. Lane 2: probe 2, Xba I, normal 9.4 kb band. Lane 3: probe 2, Kpn I, normal 20 kb band. Lane 4: probe 1, Bgl II, normal 4.9 kb band, abnormal 8.0 kb band. Lane 5: probe 1, Eco RI, normal 17.1 kb band, abnormal 7 kb band.](B)
This conclusion was supported by the finding that probe 2 in Fig 2 identified only normal bands on Southern blots of the patient TH. Since the data summarized in Fig 6 indicated that the Ph-22-bkpt in patient TH was adjacent to probe 1, it would have been expected that in the absence of deletion probe 2 would identify the 9q+ chromosome fragments. In fact, probe 2 identified only normal restriction bands on Southern blots of the patient TH (Fig 5A, lanes 1 and 2). This implies that probe 2 sequence was not present in the 9q+ or Ph chromosome. The restriction data on the 9q+ chromosome generated by the chromosome 9 probe indicate that the 9q+22-bkpt is located roughly 8 kb 3' to the bcr. As shown in Fig 7, taken together, these data indicate that a region of chromosome 22 extending from probe 1 to the 9q+22-bkpt is lost during the translocation. This deleted fragment is 8 to 10 kb.

Similar results are shown for another patient in Fig 5B. The more 5' bcr probe clearly identifies a breakpoint within the bcr, whereas the 3' probe detects only normal restriction fragments. This again implies that a portion of chromosome 22 located on the 3' side of the Ph-22-bkpt was deleted during the translocation. This pattern was seen in two additional patients.

There is an additional group of four patients who show only normal restriction fragments with both chromosome 22 probes. When probes from further 5' on chromosome 22 are available it will be possible to determine whether these patients also have a Ph-22-bkpt with a 3' deletion. Though not previously observed, it is also possible that these patients have breakpoints outside the bcr. The results for the entire group of patients are presented in Table 1 and summarized in Table 2.

**DISCUSSION**

We have documented variable deletions occurring in the region of chromosome 22 that becomes part of the 9q+ chromosome. These data are consistent with the hypothesis that the physiologically significant chromosome in CML is the Ph chromosome, and that it is not crucial to conserve the breakpoint region that becomes part of the 9q+ chromosome in the 3' portion of chromosome 22. In contrast, since the new abl transcript and protein originate from the Ph chromosome, it appears vital to connect the portion of chromosome 9 carrying the abl locus to chromosome 22 at a breakpoint that preserves a specific chromosome 22 region extending 5' from within the bcr. Our results also indicate that most of the patients have a 5' chromosome 22 break that lies within the bcr despite deletions of more 3' chromosome 22 sequence. We have observed only a few CML patients whose Ph-22-bkpts may be outside the bcr. It will be important to determine the precise breakpoint in these individuals, and whether they generate abnormal abl RNA transcripts and proteins.

There are several possible explanations for the consistent generation of the Ph chromosome and its translocation in
Table 1. Probe 1 (5' Probe) and Probe 2 (3' Probe)

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<tr>
<th>Probe</th>
<th>Bgl II</th>
<th>Kpn I</th>
<th>Bam HI</th>
<th>Eco RI</th>
<th>Xba I</th>
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<td>MK (see data for probe 2)</td>
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<td>Normal fragments with the 3' probe/abnormal with the 5' probe</td>
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<td>TH</td>
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<td>ES</td>
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Table 2. Restriction Fragments Seen in Southern Blots of CML Patients Probed With Chromosome 22 Probes

| Patients with abnormal fragments identified with the 3' bcr probe | 6 |
| Normal fragments with 3' probe/abnormal with 5' probe | 4 |
| Normal fragments with 3' and 5' probes | 4* |

Numbers indicate size in kb for the fragments seen on Southern blots with the indicated enzymes and probes. These patients are summarized in Table 2. *The band is abnormal.

CML. It is possible that the 9,22 translocation is one of many chromosomal translocations that occur at random in human cells, or is one that is favored by the environment of early hematopoietic precursors. The environment established by previous changes within the abnormal hematopoietic stem cell might provide a selective advantage so that a clone of cells with this translocation enlarges and results in the leukemia. There may be earlier events, and also perhaps subsequent events, in addition to the formation of the Ph chromosome, that are required for the appearance of the phenotype that we recognize as CML.19,20 The question is to determine how these other events relate to the 9,22 translocation. Our data suggesting that translocation of the abl locus to a point within the bcr is crucial for the appearance of the CML phenotype emphasizes the potential etiologic role of this event. A role of the bcr in this translocation is also supported by observations that a DNAase I hypersensitive site is present 5' to the bcr in the CML-derived cell line K562 (Mears JG, submitted). Taken together, the results support the hypothesis that in the formation of the Ph chromosome abl must to be translocated to a specific transcriptionally active region of chromosome 22. Further investigation of the precise molecular changes occurring during this translo-
tion should provide additional insight into the involvement of the abl gene in the generation of this leukemia.

We have shown that deletion is a frequent occurrence during the 9,22 translocation that creates the Ph chromosome in CML. Despite the loss of as much as 8 to 10 kb of chromosome 22 sequence in the case that is best characterized, some mechanism has operated to maintain the Ph-22-bkpt within the bcr. To date, karyotype analysis has been the method used to detect the 9,22 translocation. There are situations in which this method is inadequate, as when the patient sample does not provide interpretable metaphases. There are also three reported cases in which a 9,22 translocation was documented by Southern blotting even in the absence of an identifiable Ph chromosome. In these situations, it would be advantageous to use Southern blotting rather than karyotype analysis to identify the translocation. The frequent occurrence of deletion during this translocation complicates the interpretation of such restriction analysis.

REFERENCES


From our data, a probe from the 3' portion of the bcr is inadequate in a significant number of patients. The most desirable probe for detecting the rearrangement would be a chromosome 22 fragment located 5' of all Ph chromosome breakpoints. If all patients have a chromosome 22 breakpoint within the 5.8 kb bcr, then a fragment from the 5' end of the bcr would be an acceptable probe. More data on the location of chromosome 22 breakpoints and the extent of deletion in patients with a Ph chromosome will be required to determine what probe will be most reliable for reproducibly detecting the 9,22 translocation.

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