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

By Daniel W. Popenoe, Kim Schaefer-Rego, J. Gregory Mears, Arthur Bank, and David Leibowitz

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]

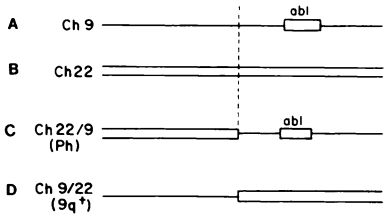

Fig 1. Schematic representation of the 9,22 translocation products and the production of the Ph chromosome. (A) Normal chromosome 9. The dashed line indicates the chromosomal breakpoint. The *v-abl* homologous region of the *abl* oncogene is indicated on chromosome 9. The breakpoint location on chromosome 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.

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.[5] 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

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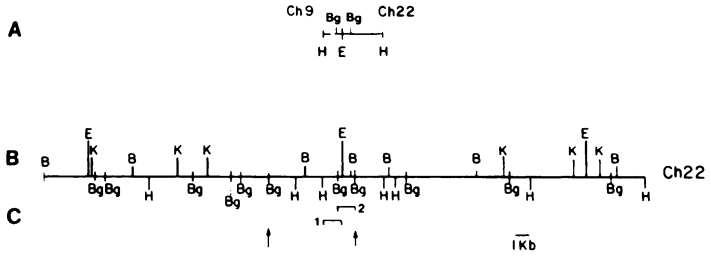

Fig 2. (A) The 9q+ chromosome junction clone from FP, a CML patient[5] is at the top. The 5′ end of the junction clone, shown in A, is sequence from chromosome 9, the 3′ end is chromosome 22 sequence. (B) A map of chromosome 22 derived from reference 7 and work in this laboratory. The Bgl II fragment of the clone in A was used to screen a normal human recombinant library to generate chromosome 22 clones and the fragments identified in C were used as probes. (C) Fragments of chromosome 22 used as probes. The Hind III/Eco RI fragment, #1, is referred to as the 5′ probe. The Bgl II fragment, #2, is the 3′ probe. The vertical arrows indicate the bcr as defined in Groffen et al.[7]

fragment of chromosome 22 to analyze the patients' breakpoints. Restriction mapping of the patient FP, described in an earlier publication,[5] had shown that the 9q+ chromosome translocation junction point was contained within a 5.0 kb Hind III fragment (Fig 2). A recombinant DNA library was constructed in the phage vector charon 21a, and approximately 1×10^6 phage were screened using a Hind III-Eco RI fragment of chromosome 9 located just 5′ of the *v-abl* homologous region.[5] 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.

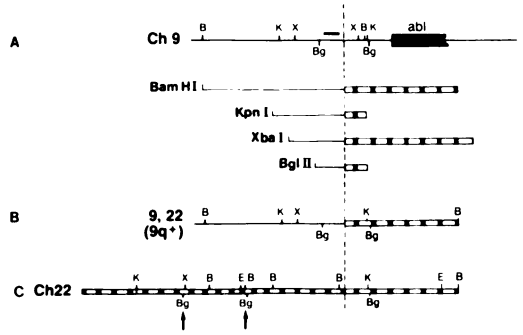

Fig 3. Restriction analysis of the 9q+ chromosome from patient TH (from reference 5). (A) The top line is a map of chromosome 9, the region labelled *abl* indicates the 5′ end of the *v-abl* homologous region; the solid black bar above the restriction map indicates a fragment of chromosome 9 used as a probe on Southern blots. The lines labelled Bam HI, Kpn I, Xba I, and Bgl II depict the abnormal restriction fragments identified on Southern blots by the chromosome 9 probe when DNA from patient TH is digested with the respective enzymes. The sizes of the abnormal fragments are Bam HI, 24 kb; Kpn I, 5.4 kb; Xba I, 15 kb; Bgl II, 4.4 kb.[6] These new restriction fragments are from the 9q+ chromosome. The thin black line indicates the portion of the fragment that is from chromosome 9, the hatched portion of the line represents the fragment derived from chromosome 22. (B) Restriction map of the 9q+ chromosome based upon the restriction data from A. The chromosome 9 probe localizes the breakpoint on chromosome 9. The size of the abnormal restriction fragments is known from Southern blots. The known restriction map of normal chromosome 22 in C, together with these restriction data make it possible to generate the restriction map of the chromosome 22 portion of the 9q+ chromosome. (C) The vertical dashed line indicates the chromosomal breakpoint. The alignment of the 9q+ chromosome with the normal chromosome 22 map indicates that the portion of chromosome 22 that became part of the 9q+ chromosome terminated roughly 8 to 10 kb 3′ of the bcr (shown by the vertical arrows below C at Bgl II sites). This chromosome 22 breakpoint which joins the 9q+ chromosome will be referred to as the 9q+-22-bkpt. The letters indicate restriction sites for the enzymes Bam HI, Kpn I, Xba I, Bgl II, and Eco RI.

RESULTS

Using a *v-abl* fragment as a probe to screen a normal human recombinant library permitted the isolation of human

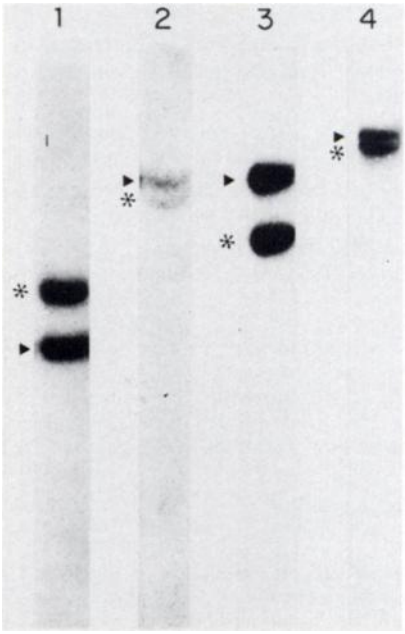

Fig 4. Southern blots of 2 patients and 1 CML cell line probed with the more 3′ bcr probe, probe 2. In each sample, a normal band is seen, indicated by an arrowhead, plus an additional abnormal band indicated by an asterisk. This abnormal band represents a translocation junction fragment (Figs 1 and 2). Lane 1: patient MK, enzyme Hind III, normal 4.2 kb band, abnormal 6.5 kb band. Lane 2: cell line EM2, Xba I, normal 9.4 kb band, abnormal 8.0 kb band. Lane 3: patient FP, Xba I, normal 9.4 kb band, abnormal 5.4 kb band. Lane 4: patient FP, Kpn I, normal 20 kb band, abnormal 18 kb band.

c-abl fragments which were then employed as probes to examine Southern blots of CML patients.[5] These probes identified two patients whose chromosome 9 breakpoints were within 4 to 6 kb of the *v-abl* homologous region.[5] 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 9,22 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.

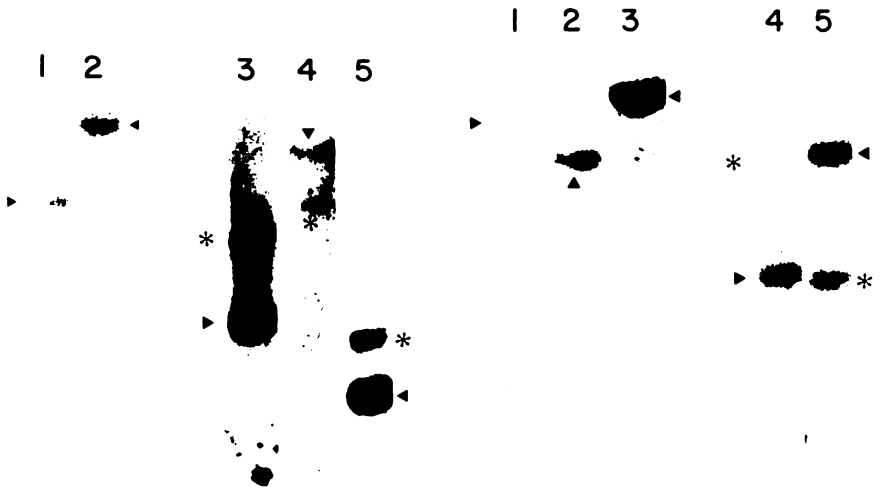

Fig 5. (A) Southern blots of a single patient, TH, representing the group of patients who showed only normal bands with the more 3′ chromosome 22 probe, but were found to have breakpoints within the bcr when probed with the more 5′ chromosome 22 probe. Arrowhead indicates normal band; asterisk indicates 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. (B) 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.

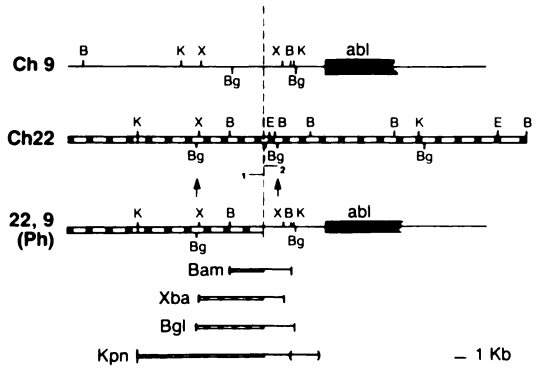

Fig 6. Ph chromosome translocation for the patient TH. Line 1: Map of normal chromosome 9. Solid black region represents the 5′ end of the region homologous to *v-abl*. Vertical dashed line represents location of chromosome break. Line 2: Map of normal chromosome 22. Vertical arrows below the line indicate the extent of the bcr. Horizontal lines labelled 1 and 2 represent chromosome 22 fragments used as probes. Line 3: Map of the Ph chromosome for patient TH. Hatched region is sequence from chromosome 22, solid line is from chromosome 9. The location of the chromosome 22 breakage which joined the Ph chromosome (Ph-22-bkpt) was determined by the appearance of abnormal restriction bands in Southern blots of DNA from patient TH. The probe for these blots was probe 1, and the abnormal bands are indicated below Line 3. The restriction enzyme that generated each new band is shown to the left of the band. The 5′ portion of each fragment is from chromosome 22, as is indicated by the hatched line; the 3′ portion is from chromosome 9, as indicated by the solid line. Three of these abnormal bands are seen in the blots in Fig 5A: Lane 5, Bam HI, 4.4 kb abnormal fragment; Lane 3, Bgl II, 7.1 kb abnormal fragment; Lane 4, Kpn I, 13 kb abnormal fragment. The length of the Kpn fragment has varied slightly on different Southern blots.

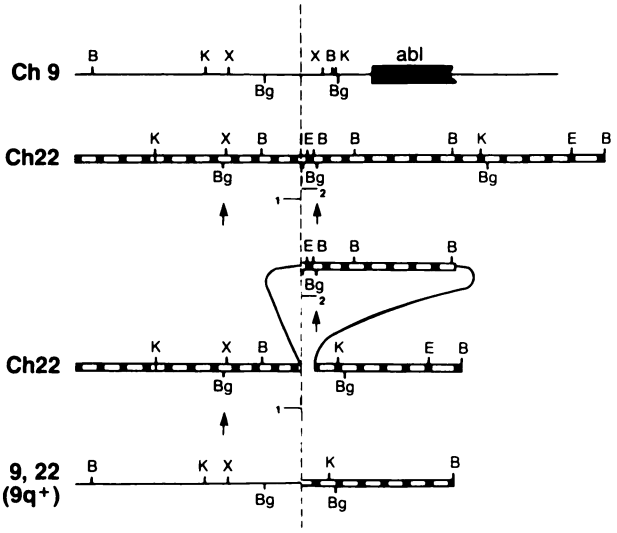

Fig 7. Diagram of deletion during the 9,22 translocation in patient TH. Lines 1 and 2 are as seen in Fig 6. The vertical dashed line indicates the point of chromosome breakage for the creation of the Ph chromosome, as shown in Fig 6. Line 4 is the restriction map for the 9q+ chromosome in TH, as illustrated in Fig 3. Line 3: Illustrates the region of chromosome 22 that was lost during this translocation. The deleted region includes that represented by probe 2. Since probe 2 sequence is not present in the 9q+ chromosome, Southern blots with probe 2 will show only normal restriction fragments, as shown for patient TH in Fig 5A, lanes 1–2, and for a second similar patient in 5B.

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)

	Probe 1				
	Bgl II	Kpn I	Bam HI	Eco RI	Xba I
Abnormal fragments identified with the 3′ bcr probe					
MK (see data for probe 2)					
EM2 (see data for probe 2)					
FP (see data for probe 2)					
MP (see data for probe 2)					
GP (see data for probe 2)					
DA (see data for probe 2)					
Normal fragments with the 3′ probe/abnormal with the 5′ probe					
TH	4.9/7.1*	20/13*	3.2/4.4*		
ES	4.9/8.0*			17.1/7*	
Co	4.9		3.2/7*	17.1/27*	
Bn	4.9/8.4*				
Normal fragments with 3′ and 5′ probes					
St	4.9		3.2		
HO	4.9				
CD	4.9			17.1	9.4
Li	4.9			17.1	9.4

	Probe 2				
	Hind III	Xba I	Kpn I	Eco RI	Bam HI
Abnormal fragments identified with the 3′ bcr probe					
MK	4.2/6.5*	9.4/3.8*			3.2/2.5/19*
EM2		9.4/8.0*			
FP		9.4/5.4*	20/18*		
MP	4.2/3.6*				3.2/2.5/1.9*
GP			20/18*		3.2/2.5/2.8*
DA		9.4/12.2*	20/12.5*		
Normal fragments with the 3′ probe/abnormal with the 5′ probe					
TH	4.2	9.4		16.7	
ES		9.4	20	16.7	3.2/2.5
Co					3.2/2.5
Bn	4.2	9.4	20		3.2/2.5
Normal fragments with the 3′ and 5′ probe					
St	4.2				3.2/2.5
HO		9.4	20	16.7	
CD		9.4	20	16.7	3.2/2.5
Li				16.7	

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 transloca-

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*

*Some of these patients may have 5′ breakpoints within the bcr that will become apparent with more 5′ chromosome 22 probes.

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.[21-23] 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. 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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