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Mapping of four distinct *BCR*-related loci to chromosome region 22q11: Order of *BCR* loci relative to chronic myelogenous leukemia and acute lymphoblastic leukemia breakpoints

(*ABL*/chromosome translocation/Philadelphia chromosome)

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ABSTRACT **A probe derived from the 3′ region of the *BCR* gene (breakpoint cluster region gene) detects four distinct loci in the human genome. One of the loci corresponds to the complete *BCR* gene, whereas the others contain a 3′ segment of the gene. After *Hin*dIII cleavage of human DNA, these four loci are detected as 23-, 19-, 13-, and 9-kilobase-pair fragments, designated *BCR4*, *BCR3*, *BCR2*, and *BCR1*, respectively, with *BCR1* deriving from the original complete *BCR* gene. All four *BCR* loci segregate 100% concordantly with human chromosome 22 in a rodent–human somatic cell hybrid panel and are located at chromosome region 22q11.2 by chromosomal *in situ* hybridization. The *BCR2* and *BCR4* loci are amplified in leukemia cell line K562 cells, indicating that they fall within the amplification unit that includes immunoglobulin λ light chain locus (*IGL*) and *ABL* locus on the K562 Philadelphia chromosome (Ph[1]); additionally, in chronic myelogenous leukemia-derived mouse–human hybrids retaining a Ph[1] chromosome in the absence of the 9q$^+$ and normal chromosome 22, *BCR2* and *BCR4* loci are retained, whereas the 3′ region of *BCR1* and the *BCR3* locus are lost, indicating that *BCR3* is distal to *BCR1* on chromosome 22. Similarly, in mouse–human hybrids retaining a Ph[1] chromosome derived from an acute lymphoblastic leukemia—in the absence of the 9q$^+$ and 22, only *BCR2* and *BCR4* loci are retained, indicating that the breakpoint in this acute lymphoblastic leukemia, as in chronic myelogenous leukemia, is proximal to the *BCR1* 3′ region, but distal to the *IGLC* locus and the *BCR2* and *BCR4* 3′ loci. Thus, the order of loci on chromosome 22 is centromere→*BCR2*, *BCR4*, and *IGL*→*BCR1*→*BCR3*→*SIS*, possibly eliminating *BCR2* and *BCR4* loci as candidate targets for juxtaposition to the *ABL* gene in the acute lymphoblastic leukemia Ph[1] chromosome.**

The leukemic cells of >90% of chronic myelogenous leukemia (CML) patients carry the translocation t(9;22)(q34;q11) that generates the Philadelphia chromosome (Ph[1]) (1, 2). The Ph[1] breakpoints in CML are clustered on chromosome 22 band q11 in a 5.8-kilobase (kb) region designated *BCR* for breakpoint cluster region (3). The *ABL* protooncogene is translocated from chromosome 9 band q34 into *BCR* (4), and the consequence of this molecular rearrangement is the production of aberrant 8-kb ABL mRNA (5) and an abnormal fusion protein, BCR–ABL p210, with enhanced protein kinase activity (6) compared with the normal p145 ABL protein. Ph[1] is also found in ≈15–25% of patients with acute lymphoblastic leukemia (ALL) and is indistinguishable cytogenetically from the Ph[1] of CML (7, 8). In contrast to CML, *BCR* is not always involved in the translocation in Ph^{1+} ALL patients (9, 10). In at least one Ph^{1+} ALL case in which *BCR* was not rearranged (Ph^{1+}, *BCR*$^-$ cases), the breakpoint in 22q11 was distal (3′) to the immunoglobulin gene for the constant region of the λ light chain, *IGLC*, and proximal (5′) to *BCR* (10). Recently, several groups have observed a novel *ABL*-encoded protein product, p190, in Ph^{1+}, *BCR*$^-$ ALL cases (8, 11, 12). The appearance of the p190 protein correlates with the expression of unusual ABL mRNA (8, 11, 12). It has been proposed that Ph^{1+}, *BCR*$^+$ (with rearranged *BCR*) ALLs are lymphoid blast crises following a clinically silent chronic phase of CML arising in multipotential stem cells, whereas Ph^{1+}, *BCR*$^-$, p190$^+$ cases are *de novo* ALLs arising from more restricted precursors (9, 12).

Thus, the previous studies on CML and ALL have demonstrated that similar chromosome abnormalities have resulted in (*i*) juxtaposition of *ABL* to the *BCR* gene resulting in the fused BCR–ABL mRNA and protein in CML and ALL with rearranged *BCR*, and (*ii*) juxtaposition of *ABL* to other, as yet unidentified, sequences, resulting in a novel ABL mRNA and p190 protein product in Ph^{1+}, *BCR*$^-$ ALL cases.

Some of us (B.L., E.F., E.S., E.C., unpublished work) have recently found that, in addition to the original *BCR* gene associated with CML, the human genome contains three *BCR*-related genes containing the 3′ region of *BCR*. Nucleotide sequencing indicated very high homology, both in exons and introns between the original *BCR* gene and the one *BCR*-related gene thus far analyzed. Because one or more of these new *BCR* loci could be involved in the pathogenesis of Ph^{1+}, *BCR*$^-$ ALL, or other malignancies, we have determined the chromosomal location of these loci relative to ALL and CML breakpoints and the original *BCR* gene.

MATERIALS AND METHODS

Molecular Probes. The probe used to detect *BCR*-related loci on Southern blots was a BCR cDNA-derived 162-base-pair (bp) *Sau*3A fragment spanning nucleotides 1028–1190 (13) contained within exons C-2 and C-3 of Fig. 1. For chromosomal *in situ* hybridization, a 725-bp *Pst* I fragment from the 3′ region of BCR cDNA, spanning the 162-bp *Sau*3A fragment described above, was used. To confirm presence or absence of specific regions of human chromosome 22 in somatic cell hybrids, an 8-kb human *IGLC* genomic *Eco*RI fragment (15) and a 1.7-kb *Bam*HI fragment containing the 3′ exon of the human *SIS* gene (16) were used. Probes for Southern blots were radiolabeled by nick translation using all four [α-^{32}P] deoxynucleotide triphosphates (dNTPs) to a specific activity of ≈1×10^9 cpm/μg. For chromosomal *in*

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Abbreviations: CML, chronic myelogenous leukemia(s); ALL, acute lymphoblastic leukemia(s); *BCR*, breakpoint cluster region gene; Ph[1], Philadelphia chromosome; *BCR*$^-$, unrearranged *BCR*; *BCR*$^+$, rearranged *BCR*; *IGLC*, human Ig λ light chain constant region gene.

FIG. 1. Physical maps of the 3′ region of the *BCR* gene (top line) and of two *BCR*-related genes. Phages were isolated from a genomic library of human fetal liver DNA (14), or from a library constructed from white blood cells of a CML patient (λ34, λ36). Exons C-1 up to C-7 correspond to the last seven exons of the original *BCR* gene. H, E, Bg, and B correspond to sites for the enzymes *Hin*dIII, *Eco*RI, *Bgl* II, and *Bam*HI, respectively. ○, *Sac* I; ●, *Hin*cII; ▽, *Pst* I; and ▼, *Pvu* II. Dashed lines represent regions not mapped with the last four enzymes.

situ hybridization, probes were nick-translated using all four [^{3}H]dNTPs to a specific activity of 4×10^7 cpm/μg.

Cells. Isolation, propagation, and characterization of parental cells and somatic cell hybrids used in this study have been described (10, 16–20). Hybrids were characterized for expression of enzyme markers assigned to each of the human chromosomes (21). Some hybrid clones were karyotyped by trypsin/Giemsa and/or G11-banding methods as described (21). In addition, the presence of specific human chromosomes in many of the mouse–human hybrids has been confirmed by DNA hybridization using probes for genes assigned to specific human chromosomes (20).

Chromosomes or partial chromosomes in most of the hybrid cells used in this study are diagrammatically depicted in Fig. 2. Partial chromosomal regions present in hybrids were defined by chromosomal banding methods and/or by determining presence or absence in hybrid DNA of specific gene sequences that had been previously localized to specific chromosome regions.

Southern Blot Analysis. DNAs from human peripheral blood lymphocytes or human cell lines, mouse cell lines, and rodent–human hybrid cell lines were extracted by cell lysis, proteinase K digestion, phenol extraction, and ethanol precipitation. Cellular DNAs were digested with an excess of

FIG. 2. Presence of human *BCR* loci in a panel of 25 rodent–human hybrids. ■, Hybrid named in the left column contains the chromosome indicated in the upper row; ◪, presence of the long arm (or a part of the long arm, indicated by a smaller fraction of stippling) of the chromosome shown above the column; ◩, presence of the short arm (or partial short arm) of the chromosome listed above the column; and □, absence of the chromosome listed above the column. The column for chromosome 22 is boldly outlined and stippled to highlight correlation of presence of this chromosome (or region of this chromosome) with presence of the four *BCR* loci (*BCR1*, *BCR2*, *BCR3*, and *BCR4*). The pattern of retention of the four *BCR* loci in the panel is shown in the four columns to the right of the figure, where presence of a locus in the hybrid is indicated by a box enclosing a plus sign, and absence of the locus is indicated by a minus sign.

restriction enzyme *Hin*dIII, sized in 0.8% agarose gels, and transferred to nitrocellulose filters as described by Southern (22). Hybridization was done in 5× standard saline citrate (SSC; 1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0)/0.2 mg of sonicated salmon sperm DNA per ml/1× Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone) at 65°C for 15 hr. After hybridization, filters were washed (0.1× SSC, 0.1% NaDodSO$_4$, 65°C) and exposed to Kodak XAR-5 film with intensifying screens.

Chromosomal *in Situ* Hybridization. Metaphase chromosome preparations were obtained by culturing peripheral blood lymphocytes from a normal male subject (46, XY) for 96 hr in RPMI medium supplemented with 15% fetal bovine serum. Cultures were harvested by standard procedure.

Probes were radiolabeled with ^{3}H to a specific activity of 4×10^7 cpm per μg of DNA. *In situ* hybridization was done using a modification of the standard protocol (23, 24) as described (18). Grains situated on nonoverlapping chromosome regions were counted and scored.

RESULTS

The 3′ region of the CML-associated *BCR* gene was cloned in bacteriophages λ40 and λ87, and the corresponding DNAs from two *BCR*-related loci were cloned in phages λ31, λ36, λ34, and λ24, respectively (Fig. 1). The three loci shown here share the last seven exons and six introns of *BCR* as well as some sequences upstream and downstream of it. Sequences 5′ of the λ87 *Hin*dIII site at 10 kb vary completely from the sequences 5′ of the *Pvu* II site located at 10.8 kb on phage λ36 and at 12 kb on phage λ24. The three *BCR*-related loci, but not the original *BCR* gene, hybridize with a *Bgl* II–*Ava* I fragment located at 9.6–9.8 kb of phage λ36. This suggests that the three loci originated from the original *BCR* gene by insertion of the 3′ *BCR* region into another locus followed by further duplication of the new locus.

To determine chromosomal location of *BCR*-related loci, DNAs from a panel of 25 rodent–human somatic cell hybrids retaining overlapping subsets of human chromosome regions (10, 17–20, 25) (see Fig. 2) were tested for retention of *BCR* loci by Southern blot analysis. Restriction enzyme *Hin*dIII-cleaved parental and hybrid DNAs were fractionated on agarose gels, transferred to nitrocellulose, and hybridized to the radiolabeled 3′ *BCR* probe, a 162-bp *Sau*3A fragment from the 3′ end of *BCR* cDNA.

This probe detects the four *BCR* loci as *Hin*dIII fragments of 23, 19, 13, and 9 kb, designated *BCR4*, *BCR3*, *BCR2*, and *BCR1*, respectively. *BCR1* corresponds to the original *BCR* gene. The same fragments were detected by several other exonic and intronic probes (data not shown). Segregation of the *BCR* loci in the hybrid panel is exemplified by the results shown in Fig. 3. Fig. 3 *a* and *b* show the long and short exposure of a filter containing mouse- (lanes 1), human K562- (lanes 2), and human ML3- (lanes 3) derived DNAs. Lanes 1 appear negative because mouse *BCR* cross-hybridizing bands are of lower M_r and have been cut off in this photograph. Lanes 2 demonstrate amplification of *BCR4* (23 kbp) and *BCR2* (13 kbp) loci in K562 DNA, derived from a CML (26) known to have amplified *ABL* and *IGL* loci on the Ph[1] (27). The signals for *BCR2* and *BCR4* loci of ML3, an acute myelocytic leukemia-derived cell line (28) (lanes 3), are less intense than the other two *BCR* loci. The meaning of such variability in relative intensity of some *BCR* loci relative to the others, which was also observed in DNA derived from some other human cell lines, is not yet clear. Lanes 4–17 (Fig. 3*c*) contain somatic cell hybrid DNAs with and without human chromosome 22 (see Fig. 2 and legend to Fig. 3 for chromosomes retained in the hybrids); the first notable point is that each hybrid lane either retains all *BCR* loci (Fig. 3*c*, lanes 4, 5, 9, 13, 14, 16, 17) or none of them (Fig. 3*c*, lanes 6–8, 10–12, 15), indicating that the four *BCR* loci, which always segregate together, are linked on a single chromosome, chromosome 22. The remainder of the hybrid DNAs bore out this pattern; results are summarized in Fig. 2. Thus, chromosome 22 carries three *BCR*-related loci in addition to the original *BCR*.

Linkage of all *BCR*-related genes to chromosome 22 was confirmed by *in situ* hybridization of ^{3}H-labeled *BCR* 3′ cDNA probe to metaphase chromosomes from peripheral blood lymphocytes of a normal male. After autoradiography, metaphase spreads were analyzed for grain localization. About 21% of all grains were located on chromosome 22. Over 98% of the grains on chromosome 22 were between 22q11 and 22q12, with most grains at 22q11.2. A histogram depicting the silver grain distribution along the human chromosomes is shown in Fig. 4. The long arm of chromosome 22 represents ≈1.3% of the haploid genome, and our observation that more than 20% of the human *BCR* 3′ probe hybridization was localized to this region is highly significant ($P < 0.001$). Thus, the hybridization localizes the *BCR* 3′ genes to the region between 22q11 and 22q12, most likely at band 22q11.2, indicating all *BCR* 3′ genes are clustered in this region at the cytological level.

To determine the relative order of the *BCR* loci on chromosome 22, we took advantage of Ph^{1+} ALL- and CML-derived mouse–human hybrids that were previously

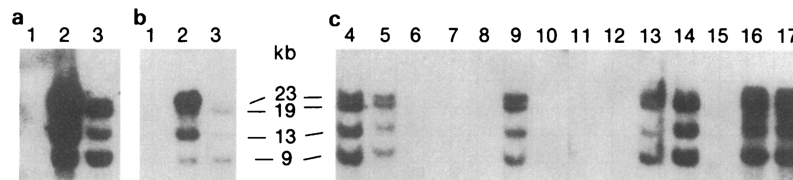

FIG. 3. The four *BCR* loci are linked on human chromosome 22 proximal to the *SIS* locus. DNA (≈10 μg) from mouse cell line (*a* and *b*, lanes 1); human K562 CML cell line (*a* and *b*, lanes 2); human ML3 acute myelocytic leukemia cell line (*a* and *b*, lanes 3); hybrid 8c retaining chromosomes 4, 9, 10, 12, 14, 15, 17, 21, 22 (*c*, lane 4); hybrid D20 retaining 5, 14, 17, 18, 20, 22, X (*c*, lane 5); hybrid DE7 retaining 1, 4, 6–8, 10–12, 14–16, 19–21, X, Y (*c*, lane 6); hybrid AB3 retaining 6, 14q11→14qter, 20pter→20q13, X (*c*, lane 7); hybrid S4 retaining 1, 3–6, 10, 11, 14, 17, X (*c*, lane 8); hybrid, 3c-9 retaining 1, 4q, part of 6, 12, 14, 17, 22, X (*c*, lane 9); hybrid GL-3a retaining part of 3, 4, 6, 7, 10, 12q, 14, 15, 17–20 (*c*, lane 10); hybrid AA3 retaining 4p, 18, X (*c*, lane 11); hybrid B2 retaining 4p, 6, 12, 20, X (*c*, lane 12); hybrid 570 retaining 10–12, 14, 15pter→15q22, 17q21→17qter, 22 (*c*, lane 13); hybrid S5 retaining 3, 5–7, 9, 13, 15, 17, 18, 22pter→22q11, X (*c*, lane 14); hybrid N9 retaining 6, 7, part of 17q, 21 (*c*, lane 15); hybrid 77-5 retaining 1, 3, 4p, 5–9, 10q, 13, 14, 17, 18, 20, 22, X (*c*, lane 16); and hybrid 77-30l retaining 3, 4p, 5, 9, 10q, 13, 14, 17, 20, 22, X (*c*, lane 17) was digested with restriction enzyme *Hin*dIII, fractionated on agarose, blotted to nitrocellulose, and hybridized to radiolabeled *BCR* 3′ probe. Molecular size of *BCR1* (9-kbp), *BCR2* (13-kbp), *BCR3* (19-kbp), and *BCR4* (23-kbp) *Hin*dIII fragments is shown between panels *b* and *c*. All four *BCR*-related fragments are present in all hybrids that retain a normal chromosome 22 and one hybrid, S5, which retains a partial chromosome 22; this hybrid, S5 (*c*, lane 14), retains *IGLC* sequences but does not retain *SIS* sequences (data not shown). All hybrids that have lost chromosome 22 have also lost all four *BCR*-related loci.

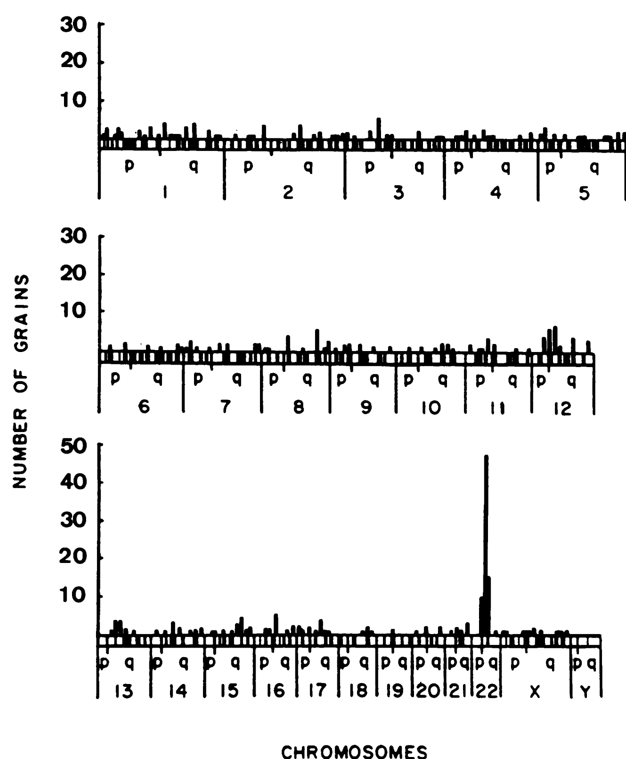

FIG. 4. Localization of *BCR*-related loci in the human genome by *in situ* hybridization analysis. Diagram showing the distribution of 344 autoradiographic grains on 80 metaphases. The abscissa represents the chromosome banding pattern of each human chromosome in relative size proportions; the ordinate shows the number and location of silver grains. Twenty-one percent of grains are on 22q; most 22q grains are in region 22q11.2.

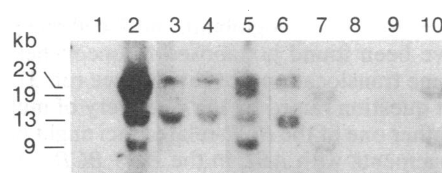

FIG. 5. Regional mapping of *BCR*-related loci relative to ALL, CML, and BL2 [Burkitt lymphoma variant t(8;22)(q24;q11)] breakpoints. DNA (≈10 μg) from mouse cell line (lane 1); human K562 CML cell line (lane 2); hybrid AA2-A2 retaining Ph^1 (22pter→22q11::9q34→9qter) from ALL in the absence of normal 22 and 22q11→22qter (lane 3); hybrid AA2 retaining Ph^1 (same Ph^1 as hybrid AA2-A2) in the absence of normal 22 and 22q11→22qter (lane 4); ALL patient 4 parent of AA2 hybrids (lane 5); mouse × Bv173 (CML) hybrid retaining Ph^1 ($22q^-$) in the absence of normal 22 and $9q^+$ (9pter→9q34::22q11→22qter) (lane 6); hybrid 1-23-5 retaining $8q^+$ (8pter→8q24 :: 22q11→22qter) from variant Burkitt lymphoma, BL2, with t(8;22)(q24;q11), in the absence of normal 22 and $22q^-$ (22pter→22q11::8q24→8qter) (lane 7); hybrid N9 retaining no part of chromosome 22 (lane 8); hybrid 275S retaining no part of chromosome 22 (lane 9); and hybrid 1-23-12 retaining an $8q^+$ from Burkitt lymphoma BL2 in the absence of $22q^-$ and normal 22 (lane 10) was cleaved with *Hin*dIII, electrophoresed, blotted, and hybridized to the 162-bp 3′ *BCR* probe.

characterized (10, 25); additionally, we have used hybrids retaining an $8q^+$ (8pter→8q24::22q11→22qter) (17) derived from a Burkitt lymphoma (BL2) carrying a t(8;22)(8q24;q11) for which the breakpoint has been cloned and characterized (29). These hybrids have been tested for retention of *BCR* loci by Southern blotting analysis as depicted in Fig. 5 and summarized in Table 1. Lanes 3 and 4 of Fig. 5 contain DNA from hybrids retaining a $22q^-$ (22pter→22q11::9q34→9qter, from Ph^{1+}, BCR^- ALL patient 4 as described in ref. 10) but not 22 or $9q^+$. These hybrids retain the *BCR2* and *BCR4* 3′ loci but have lost the *BCR1* and *BCR3* loci; thus the chromosome 22 breakpoint of the parental ALL, which has been shown to be distal to the *IGLC* locus (10), is also distal to the *BCR2* and *BCR4* 3′ loci. Lane 6 contains DNA derived from a Bv173 (30) hybrid retaining a $22q^-$ (from a Ph^{1+}, BCR^+ CML) in the absence of the $9q^+$ and normal 22 (25). This hybrid also retains *BCR2* and *BCR4* loci but has lost the 9-kbp 3′ *BCR1* (as expected for a BCR^+ CML) and the *BCR3* (19-kbp *Hin*dIII fragment), so the *BCR3* locus is distal to the *BCR1* locus. Lanes 7 and 10 (Fig. 5), containing DNA from BL2 × mouse hybrids that retain an $8q^+$ from BL2 in which the chromosome 22 break is in an Ig gene for λ light chain variable region, *IGLV* (29), show only the *BCR1* and *BCR3* loci, indicating that the *BCR2* and *BCR4* loci may be within or proximal to the *IGLV* locus. A summary of all data considered in the regional mapping of the four *BCR* loci is shown in Table 1.

DISCUSSION

The unexpected finding that intronic as well as exonic probes from the 3′ region of *BCR* genomic or cDNA clones, respectively, reveal the presence of three additional *BCR*-related loci provokes immediate analogy to the only other

Table 1. Segregation of *BCR*-related loci in hybrids retaining defined regions of chromosome 22

	Chromosome region retained			Chromosome 22-linked genes retained					
					BCR				
Hybrid	22q11→22qter	22	22pter→22q11	*IGLC*	1	2	3	4	*SIS*
Ph^{1+} ALL-derived*									
AA2	−	−	+	+	−	+	−	+	−
AA2-A2	−	−	+	+	−	+	−	+	−
Ph^{1+} CML-derived†									
260-3-12-3	−	−	+	+	−	+	−	+	−
BL t(8;22)-derived‡									
1-23-12	+	−	−	+	+	−	+	−	+
1-23-5	+	−	−	+	+	−	+	−	+
Normal human-derived									
S5	−	−	+	+	+	+	+	+	−

*Parental ALL patient 4 and hybrids were previously described (10).
†Bv173 hybrids were previously described (25).
‡BL2 Burkitt lymphoma hybrids were previously described (17) and the breakpoint within *IGLV* has been cloned and sequenced (29).

types of gene loci, immunoglobulin and T-cell receptor genes, which have been found juxtaposed to oncogenes following chromosome translocation (for review, see ref. 31). Another prominent question raised by the discovery of multiple *BCR* loci is whether one of the *BCR*-related loci might be involved in rearrangements with *ABL* in the Ph^{1+} BCR^- ALLs. This possibility would be ruled out if the *BCR*-related loci were not located in chromosome region 22q11. Accordingly, an important early priority was to determine the chromosomal location of the *BCR*-related loci.

Initial screening of DNAs from a panel of rodent–human hybrids retaining overlapping subsets of human chromosomes demonstrated that the four *BCR*-related genes were linked on human chromosome 22. Chromosomal *in situ* hybridization using a labeled probe from the region common to all *BCR* loci confirmed that this probe hybridized only to human chromosome region 22q11. Consequently, the three *BCR*-related loci were mapped on chromosome 22 relative to CML and ALL breakpoints using somatic cell hybrids retaining Ph^1 and other relevant chromosomes. We concluded from the combined mapping and *in situ* and regional data that (*i*) all four *BCR* loci are proximal to the *SIS* locus on chromosome 22; (*ii*) the *BCR3* locus is distal to *BCR1*, and the *BCR2* and *BCR4* loci are proximal to *BCR1*; (*iii*) in CMLs and in the ALL tumor parent of the AA2 hybrid, the chromosome 22 breakpoint is proximal to the *BCR1* 3′ region and distal to the *BCR2* and *BCR4* 3′ regions; and (*iv*) the *BCR2* and *BCR4* loci may be within or proximal to the *IGL* locus.

Finally, the fact that neither *BCR2* nor *BCR4* *Hin*dIII fragments are rearranged in ALL patient 4 (Fig. 5, lane 5) or several other Ph^{1+}, BCR^- ALLs (data not shown) and, most importantly, that the *BCR2* and *BCR4* 3′ loci remain on the Ph^1 chromosome in at least one Ph^{1+} BCR^- ALL, lends no support to the hypothesis that one of the new *BCR* loci could be involved in the Ph^1 translocation of ALLs. In fact, from the mapping of the four *BCR* loci relative to the chromosome breakpoint of the ALL patient 4 analyzed here, the only *BCR* locus that could be a candidate for juxtaposition to *ABL* is the original *BCR1* locus.

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