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ABR, an active BCR-related gene

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ABSTRACT

The human BCR gene on chromosome 22 is specifically involved in the Philadelphia translocation, t(9;22), a chromosomal rearrangement present in the leukemic cells of patients with chronic myeloid leukemia or acute lymphoblastic leukemia. In most cases, the breakpoints on chromosome 22 are found within a 5.8 kb region of DNA designated the major breakpoint cluster region (Mbcr) of the BCR gene. Hybridization experiments have indicated that the human genome contains BCR gene-related sequences. Here we report the molecular cloning of one of these loci, for which we propose the name ABR. In contrast with the other BCR-related genes studied to date, ABR represents a functionally active gene and contains exons very similar to those found within the Mbcr. Unlike the BCR gene, the ABR gene exhibits great genomic variability caused by two different variable tandem repeat regions located in two introns. All other BCR gene-related sequences isolated so far and the BCR gene itself are located on chromosome 22. In contrast, the ABR gene is located on chromosome 17p.

INTRODUCTION

The human BCR gene located on chromosome 22 at band q11 is specifically involved in chromosomal translocations associated with leukemia. All chronic myelocytic leukemia (CML) (1,2) and a percentage of acute lymphoblastic leukemia (ALL) patients have the Philadelphia (Ph) chromosome in their leukemic cells. This chromosome usually represents one product of a reciprocal translocation between chromosome 9 and 22 t(9;22) (q34;q11). For unknown reasons, the breakpoints on chromosome 22 occur with great specificity within a 5.8 kb region of DNA designated the major breakpoint cluster region (Mbcr), located within the BCR gene (3). Thus, the Mbcr represents a segment of the gene, encompassing five exons and their intervening sequences (4).

Previous experiments have indicated that the human genome contains many BCR gene-related sequences. We and others have molecularly cloned three of these loci, all of which were located on chromosome 22 q11.2 (5,6,21). None of these regions represented active BCR-related genes: all contained apparently non-functional exons and introns homologous only to the 3' end of the BCR gene. In addition to these loci, one other BCR related locus has been identified but not characterized (6). Preliminary data suggested that this locus contained sequences homologous to the exons located within the Mbcr. As the Mbcr appears to be a "hotspot" for recombination events, the putative involvement of

these related sequences in rearrangements and/or tumorigenesis is of interest. This report describes the isolation and characterization of this region, which is part of a functionally active BCR-related gene.

MATERIAL AND METHODS

DNAs and RNAs

Cell lines for DNA and RNA isolation were obtained from the ATCC and from the Mutant Cell Repository, Camden, New Jersey. DNAs were also isolated from tumor tissue obtained through the Biological Carcinogenesis Branch, DCCP. High molecular weight DNAs were isolated as described (7). RNAs were isolated from cell lines using either LiCl/urea (8) or guanidine-isothiocyanate (9).

Southern and Northern blot analysis

DNAs digested with different restriction enzymes (Bethesda Research Laboratories) were run on agarose gels and blotted according to Southern (10). RNAs (20 µg total or 10 µg poly A+) were run on 1% agarose gels in the presence of formaldehyde (11). Blots were hybridized to probes labelled by primer extension (12). Final post-hybridization washings were in 0.3 x SSC, 0.1% SDS, 0.1% sodium pyrophosphate at 65°C.

Molecular cloning and characterization of clones

DNAs (4 x 10 µg) digested with Eco RI or Bgl II were electrophoresed on 0.7 % low melting point agarose gels (IBI). Segments of the gel containing fragments of the desired size range were excised from the gel; DNAs were purified from the agarose as described (6). Genomic DNAs were ligated into λgtwes digested with Eco RI or EMBL 3 digested with Bam HI. Libraries were screened with an 0.46 kb Pst I BCR cDNA probe (6) or with an 0.4 kb Bgl II/Hind III probe from the ABR cDNA (see below). Post-hybridization washings were at 0.3 x SSC at 65°C.

Inserts from hybridizing phage were subcloned into pSK (Stratagene) or pUC and mapped. Exons were located by hybridization of ABR or BCR cDNAs to subclones (data not shown) mapped in great detail. Small hybridizing fragments were subcloned into M13 phage for sequence analysis. Sequencing was performed using the dideoxy chain termination method (13).

In situ hybridization

Karyotypically normal human metaphases were prepared from heparinized peripheral blood of a male donor, 46,XY, and from a 46, XX, lymphoblastoid cell line (GM 7387, Mutant Cell Repository). Whole blood (0.5 ml) was cultured for 72 hr at 37°C in RPMI 1640 with phytohaemagglutinin and fetal calf serum. Metaphase cells were arrested from the lymphoblastoid line 48 hr after subculturing. Cells were harvested by standard colcemid, hypotonic and fixation procedures. An 0.4 kb XhoI/Bgl II fragment (see below) subcloned into pSK (Stratagene) was nick-translated with ^{3}H-dNTPs to a specific activity

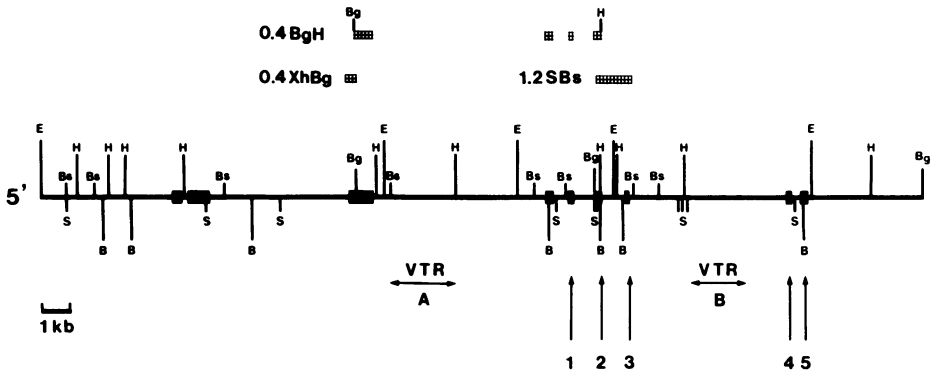

Figure 1. Restriction enzyme map of the ABR locus. The location of the probes used in this study are indicated above the restriction enzyme map with hatched boxes. Boxed areas in the map delineate the approximate position of exons; Mbcr-homologous exons 1-5 are noted with vertical arrows beneath the map. The approximate locations of the variable tandem repeats A and B are indicated with horizontal arrows. Restriction enzymes used include B= Bam HI, Bg = Bgl II, Bs = Bst EII, E = Eco RI, H = Hind III, S = Sst I.

of 1-2 x 10^7 cpm/µg and hybridized in situ to metaphase chromosomes as described (14).

RESULTS

Cloning of BCR-gene homologous locus ABR

An 0.46 kb Pst I fragment from the BCR gene cDNA is a relatively specific probe for exons within Mbcr. This fragment detects Mbcr exons 2,3,4 and 5 of the BCR gene which are distributed over three Bgl II fragments of 4.3, 3.4 and 1.1 kb. This probe also hybridizes to two Bgl II fragments of 12.0 and 6.0 kb which do not belong to the BCR gene locus (6). In our initial attempts to isolate the latter two fragments, the 0.46 Pst I probe was used to screen three different cosmid libraries. However, not a single positive was obtained even though these libraries had previously been used to isolate the entire BCR gene (3), the ABL, FES and FMS oncogenes (6,15,16) and two non-functional BCR related genes (6). This indicated, that the sequences of interest were either under-represented during the construction of the libraries or that they had been selectively lost due to the presence of unstable or "poisonous" sequences.

We therefore used a different cloning strategy. Bgl II-digested DNA was size-fractionated in the 12.0 and 6.0 kb range and libraries were constructed in EMBL 3. The 12.0 and 6.0 kb Bgl II fragments were isolated from these (unamplified) libraries. The 6.0 kb Bgl II fragment showed limited homology to the 0.46 kb Pst I probe (results not shown) and will not be discussed further. In contrast, the 12.0 kb fragment contained an apparent exon-intron organization. Fragments which hybridized to the 0.46 kb cDNA

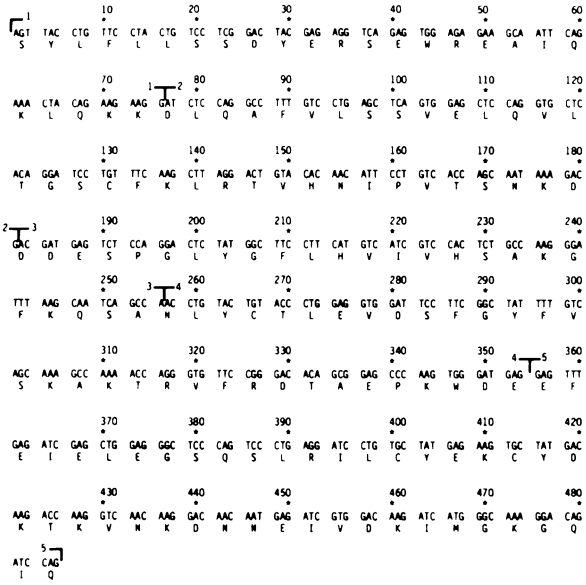

Figure 2. Nucleotide and deduced aminoacid sequence of ABR exons 1-5. The junctions between the exons are indicated in the sequence.

probe were subcloned and sequenced. The 12.0 kb Bgl II fragment contains four exons of a BCR-related gene, which are closely similar (see below) to the Mbcr exons 2,3,4 and 5. However, the 12 kb region cloned lacked Mbcr-homologous exon 1. To isolate this region, size-selected genomic libraries were constructed: using an 0.4 kb BgH probe (see below and Fig. 1) an 8.5 kb Bgl II fragment immediately 5' to the 12.0 kb Bgl II fragment and a 12.0 kb Eco RI fragment 5' to and overlapping with the 8.5 kb Bgl II fragment were cloned. Obtaining the 8.5 kb Bgl II fragment was especially laborious: the screening of 10^6 recombinant phage of a freshly prepared (unamplified) library yielded one positive. Subcloning of the insert from the phage arms produced clones of different sizes which had deleted varying amounts of the insert. In total, approximately 30 kb of the locus was cloned in phage and restriction enzyme mapped (Fig. 1). Since this locus appears to represent an active (see below) BCR-related gene, we propose the name ABR.

ABR is an actively transcribed gene

A 1.2 kb Sst I - Bst E II (1.2 SBs) probe, which contains exons 2 and 3 (see Fig. 1) was used to isolate six cDNAs from an SV-40 transformed fibroblast (17) and an A498 kidney carcinoma cDNA library. These clones varied in size from 1.3 to 7.5 kb. All clones were identical in the region encompassing exons "1-5", but differences were noted in the 5' and 3' regions (results not shown); these differences are probably caused by cloning artifacts and/or the presence of intron sequences.

As discussed above the Mbcr region of the BCR gene is of considerable interest because of its specific involvement in CML. To study this domain the region of the cDNAs homologous to Mbcr exons "1-5" was sequenced (see Fig. 2) and the intron-exon boundaries of these exons in the genomic DNA were determined. The intron-exon organization is very similar to that of Mbcr in the BCR gene (4). ABR exons "1-5" encode 25,35,25,33 and 44 aminoacid residues respectively (Fig. 2) and the splice donor and acceptor sites are conserved (not shown). The homology between the deduced aminoacid sequences of the ABR and BCR genes within Mbcr is striking (see Fig. 3): of the 162 aminoacid residues encoded by this region, 122 (75%) are identical and differences largely represent conserved changes. Thus, exons 1-5 of the ABR gene are almost identical with exons 1-5 of the major breakpoint cluster region in the BCR gene.

Two mRNAs of 4.3 and 6.0 kb are expressed at low levels from the BCR gene in almost all cell types tested (18). The 4.3 kb mRNA has been characterized completely (4,19,20), but the exact organization of the 6.0 kb mRNA remains unknown, although it appears to contain most, if not all sequences present in the 4.3 kb mRNA (20,21). To examine expression of ABR an 0.4 kb Bgl II - Hind III cDNA probe (0.4 BgH) encompassing exon "2", exon "1" and the two exons immediately 5' to these was selected (see Fig 1). Transcripts of the gene are detected in total RNA of every cell line tested (Fig. 4, left panel). ABR mRNA is generally expressed at a relatively high level, although quantitative differences between K562 (chronic myelogenous leukemia), ChaGo (bronchogenic carcinoma), A172 (glioblastoma) and HepG2 (hepatocellular carcinoma) can be noted (Fig. 4). The signal visible in total RNA resolved as two distinct species of 5.0 and 4.8 kb (see Fig. 4, right panel) in poly A+ selected RNA. Both transcripts are expressed in equal abundance in A498 (kidney carcinoma) but the 5.0 kb transcript appears predominant in A673 (rhabdomyosarcoma) and the 4.8 kb mRNA is more abundant in A549 (lung carcinoma).

The ABR gene contains variable tandem repeat regions

As discussed above, the difficulties encountered in the cloning of this region were possibly caused by unstable or "poisonous" sequences present in the DNA. In the course of characterizing the ABR locus, a number of repeat-free genomic probes were tested to confirm the restriction enzyme map as shown in Fig. 1. The 1.2 SBs probe, for example, should hybridize to Eco RI fragments of 3.4 and 7.0 kb. However, although the 3.4 kb fragment appears to be present in all DNAs (Fig. 5, lanes 1-8), the "7.0" kb fragment showed marked variability in size between DNAs and some DNAs contain two different sized "7.0" kb alleles.

The DNAs examined were from tumor specimens, raising the possibility that these heterogenously-sized bands were tumor-related. However, DNA from human umbilical cords displayed the same heterogeneity (not shown). In addition, probes from the 3' and the 5' of the "7.0" kb Eco RI fragment hybridized to the same set of variable "7.0" kb

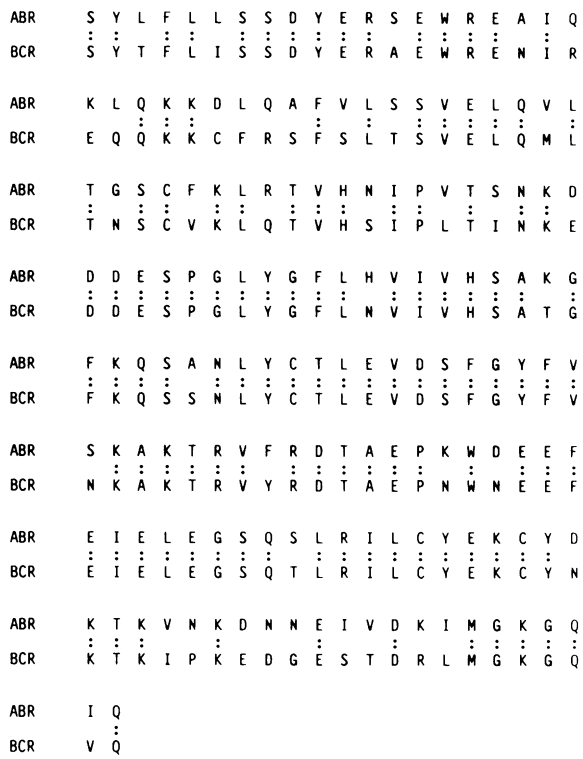

Figure 3. Comparison of the deduced aminoacid sequence of the ABR and BCR genes within the Mbcr exons 1-5.

fragments (not shown); thus, these fragments do not contain genomic abnormalities such as rearrangements and must constitute normal polymorphic variability within the population.

Further genomic mapping located the "variable" region to an area between a 5' Sst I and a 3' Xho I site (VTR B, see Fig. 1). A second variable region (VTR A, Fig. 1) was detected with the 0.4 BgH probe. This probe detects the "12.0" kb Bgl II fragment, which can vary as much as 3 kb in size between different alleles; it also hybridizes to the 5' adjacent "8.5" kb Bgl II fragment, which likewise exhibits great variability in size (Fig. 5, lanes 9-17), with alleles ranging from 7.5 to 18 kb. This variable region was located between a 5' Bst EII and a 3' Hind III site (see Fig. 1, VTR A).

Selected regions were subcloned into M13 vectors for sequence analysis. The segment sequenced of region A consists of the tandem head-to-tail repetition of a core 43 bp sequence (Fig. 6), although nucleotide changes were found in some repeat units. The difference in size between the largest and smallest allele is approximately 10.5 kb, which

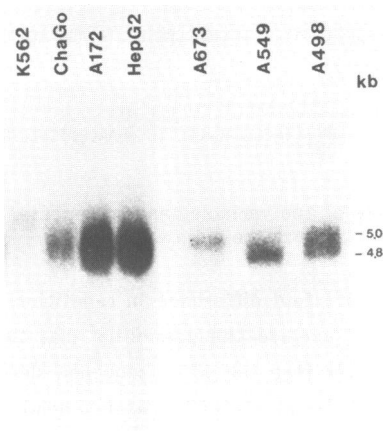

Figure 4. Northern blot analysis of ABR gene expression. RNAs used are indicated above each lane. Twenty μg of total RNA was used per lane in the left panel, whereas 10 μg of poly A+ RNA was used in the right panel. The sizes of the two ABR transcripts are indicated to the right.

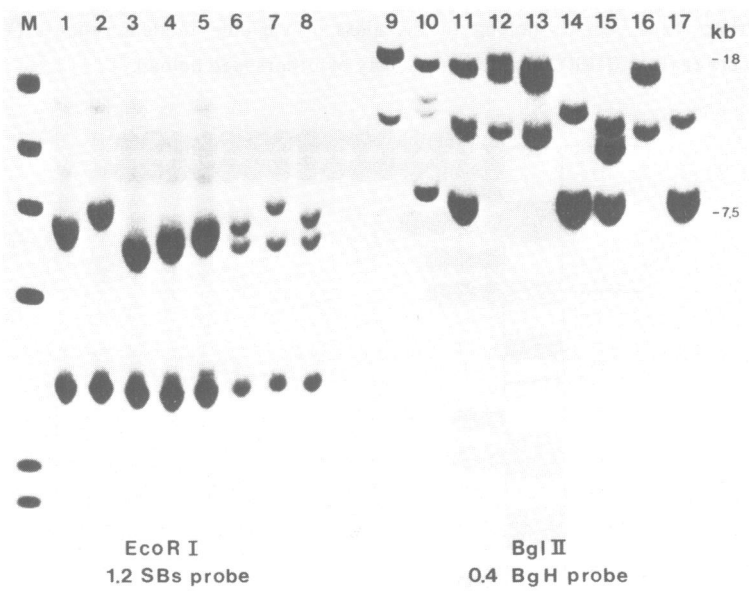

Figure 5. Southern blot analysis of the ABR gene variable tandem repeat regions. Blots were hybridized to the probes indicated below each panel; restriction enzymes are as shown. A λ/Hind III molecular weight marker is shown to the left of the first panel; the approximate molecular weights of the smallest (7.5 kb) and largest (18 kb) alleles of VTR A are indicated to the right of the second panel. DNAs used include C999 lane 1, C 481 lane 2, C080 lane 3, C011 lane 4, C987 lane 5, SK-CML-1-BN1 lane 6, SK-CML-6-BN3 lane 7, SK-CML-7B lane 8, K562 lane 9, GM 3344 lane 10, 7801Am lane 11, Amo601 lane 12, C080 lane 13, C481 lane 14, C999 lane 15, 048398 lane 16, 383-1 lane 17.

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                                        Xba I
VTR #A   CCCTCAGTTCACGTAACAACAGTGTCTAGAGGAACATGCATAG
                        A           C

                                    Bsp MII
VTR #B   GTGAGCCCTCCCCCAGACCCTTCCGGAACCCTCCTCTGACCCT
                  AT          CT
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Figure 6. Nucleotide sequence of the variable tandem repeats. Nucleotide differences within the core units are shown beneath the main sequence.

could reflect an estimated 244 - fold difference in repetition of the 43 bp sequence. However, we presently cannot exclude the possibility that this region also contains tandem repetitive sequence other than the 43 bp sequence shown here. Curiously, all segments sequenced of region B also consist of the tandem head-to-tail repetition of a 43 bp sequence, but the sequence itself is quite distinct from that of region A (Fig. 6). In addition, the difference in size between the smaller and the larger alleles is approximately 1.3 kb or less, possibly reflecting a 30-fold difference in copy number of the 43 bp repeat. Single copy probes of the 43 bp region of VTR A can be used without any problems in hybridization against Southern blotted human genomic DNA providing evidence that these repeats belong to the class of variable tandem repeats (VTR) or hypervariable regions (HVR) reported previously by others (see below).

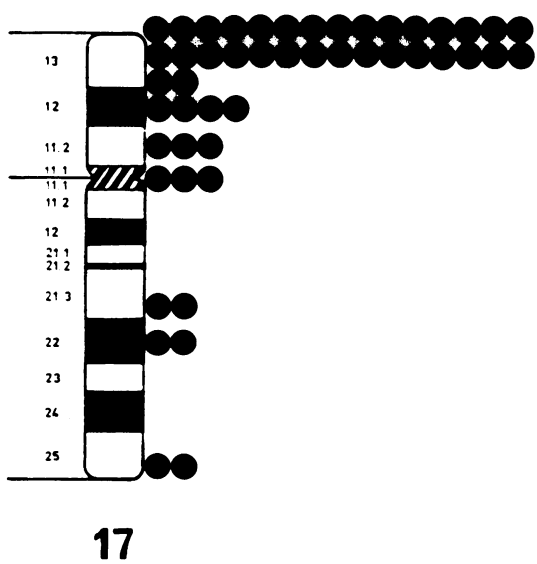

Figure 7. Chromosomal location of ABR. Combined data from two separate in situ hybridization experiments showing specific labelling on chromosome 17, band p13. A total of 377 grains were scored on 150 metaphase cells. 11.1% (42/377) of grains were on chromosome region 17p, with 76.2% (32/42) of the 17p grains localized to 17p13.

Chromosomal localization of ABR

The BCR gene as well as the non-functional BCR-related loci are all located on chromosome 22; two loci are 5' of the BCR gene and one is 3'. To determine, whether the ABR gene was located in the same vicinity or elsewhere, a repeat-free probe (0.4 XhBg, see Fig. 1) was subcloned into the plasmid vector pSK and used for chromosome in situ hybridization experiments. As shown in Fig. 7, an accumulation of grains was noted on chromosome 17p, with a peak of hybridization at 17p13.

DISCUSSION

Our results and those of others indicate that the human genome contains many BCR gene-related sequences (5,6,21). However, the ABR locus we have isolated appears to be the only one which is functional. ABR is actively transcribed in all cell lines tested and, like the BCR gene, encodes two mRNAs. These 5.0 and 4.8 kb species may be expressed in equal amounts or show a differential expression as seen in A673 (5.0 kb transcript predominates) and A549 (4.8 kb transcript predominates). Transcription from the BCR and ABR genes is not mutually exclusive: both are expressed in the same cell lines, for example in K562, A172 and A498 (unpublished results).

The ABR gene is likely to be a large gene as the incomplete region we have cloned already spans 30 kb yet does not contain the entire gene. However, it remains to be determined whether it can equal the large, 130 kb, BCR gene (22). ABR also has five small exons with a deduced aminoacid sequence very similar to exons 1-5 of the Mbcr.

ABR and BCR differ dramatically in one aspect: the BCR gene, although specifically involved in chromosomal translocations, was not particularly difficult to clone and does not appear genetically unstable. The gene does not vary in length in normal DNAs, except for a polymorphism in the first intron (23,24). In contrast, segments of the ABR gene cloned here were highly unstable when propogated in E. coli, and a large number of different-sized alleles exist in the general population. This inconstancy is caused by two variable tandem repeat regions, VTR A and B, both of which are located in introns. VTR A, in an intron 5' to the Mbcr homologous exons, is the largest source of variability. VTR B is located between Mbcr homologous exons 3 and 4. Interestingly, the location of this VTR corresponds with the Mbcr region highly prone to rearrangement in CML.

Hybridization of a VTR B probe to blots containing the cloned Mbcr region failed to detect homologous regions. This was not unexpected considering the relatively constant size of the Mbcr between individuals. At this stage, it is debatable whether the BCR gene was evolutionarily derived from the ABR gene or vice versa - the BCR gene could have deleted its VTR regions or the ABR gene could have acquired them.

Hypervariable regions have been described previously, either alone or in association with genes and the VTR regions described above fit the general pattern. For example, hypervariable regions consisting of 36, 14 and 17 bp tandem arrays have been found as

interzeta, zeta-intron and alpha-globin 3' repeats (25,26,27); minisatellites from the insulin and Ha-ras1 loci (28,29) have also been characterized and they can be used as chromosome-specific single copy probes. Abnormally high rates of genetic exchange have been observed in vivo and in vitro and it has been suggested that VTRs may promote such recombination events (30). The function, if any, of VTR sequences is unknown. They may operate as enhancer elements, or possibly feature in the organization of chromosome structure (27).

Whatever their role, it may be significant that the genetically unstable domains of the ABR gene are located in a region of a chromosome which seems to be recombination - prone: our in situ hybridization studies have localized ABR to chromosome 17, band p13. This region also contains the p53 gene (38,39). To examine, whether ABR and p53 are adjacent to each other, we have sequentially hybridized a field inversion gel electrophoresis blot of DNA digested with 6 different rare-cutting enzymes to probes from both genes; in 5 of these digests, different fragments hybridized to the probes, indicating that a close linkage is unlikely. Structural abnormalities of this region are associated with a number of clinical and malignant disorders. For example, visible or submicroscopic deletion of 17p13 [del (17) (p13)] occurs in Miller Dieker syndrome, a mental retardation syndrome (31,32,33), and loss of heterozygosity of chromosome 17p markers was recently reported in the progression of colorectal carcinoma (34). An isochromosome 17q [i(17q)] occurs frequently in a number of haematological disorders, particularly in the progressive phase of CML, and also in some solid tumours, such as pediatric brain tumours; loss of genes present on 17p may be important in these cases. Finally, band 17p13 is one of the preferentially involved breakpoint sites in variant Ph translocations of CML (35,36,37). It will obviously be of interest to determine the structure and patterns of expression of ABR in these disorders.

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REFERENCES

1. Nowell, P.C., and Hungerford, O.A. (1960). *Science*, **132**: 1497.
2. Rowley, J.D., (1973). *Nature*, **243**: 290-293.
3. Groffen, J., Stephenson, J. R., Heisterkamp, N., de Klein, A., Bartram, C.R. and Grosveld, G. (1984). *Cell* **36**: 93-99.
4. Heisterkamp, N., Stam, K., Groffen, J., de Klein, A. and Grosveld, G. (1985). *Nature*, **314**: 758-761.

5. Croce, C.M., Huebner, K., Isobe, M., Fainstain, E., Lifshitz, B., Shtivelman, E. and Canaani, E. (1987). *Proc. Natl. Acad. Sci. USA*, **84**: 7174-7178.
6. Heisterkamp, N. and Groffen, J. (1988). *Nucleic Acids Res.*, **16**: 8045-8056.
7. Heisterkamp N., Groffen, J. and Stephenson, J. R., (1983). *J. Mol. Appl. Genet.*, **2**: 57-68.
8. Stam, K., Heisterkamp, N., Grosveld, G., de Klein, A., Verma, R.S., Coleman, M., Dosik, H., and Groffen, J. (1985). *N. Engl. J. Med.*, **313**: 1429-1433.
9. Chomczynski, P., and Sacchi, N. (1987). *Anal. Biochem.* **162**: 156-159.
10. Southern, E. (1975). *J. Mol. Biol.*, **98**: 503.
11. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 202.
12. Feinberg, A.P. and Vogelstein, B. (1984). *Anal. Biochem.* **13**: 266-267.
13. Sanger, F., Nicklen, S. and Coulson, A.R. (1977). *Proc. Natl. Acad. Sci. U.S.A.*, **74**: 5463-5467.
14. Morris, C.M., and Fitzgerald, P.H. (1987). *Leuk. Res.*, **11**: 163-169.
15. Groffen, J., Heisterkamp, N., Grosveld, F., van de Ven, W.J.M., and Stephenson, J.R. (1982). *Science* **216**: 1136-1138.
16. Heisterkamp, N., Groffen, J., and Stephenson, J.R. (1983). *Virol.*, **126**: 248-258.
17. Okayama, H. and Berg, P. (1983). *Molec. Cell. Biol.*, **3**: 280-289.
18. Collins, S., Coleman, H. and Groudine, M. (1987). *Molec. Cell. Biol.* **7**: 2870-2876.
19. Mes-Masson, A-M., McLaughlin, J., Daley, G.Q., Paskind, M., and Witte, O.N. (1986). *Proc. Natl. Acad. Sci. USA.*, **83**: 9768-9772.
20. Hariharan, I.K. and Adams, J.M. (1987). *Embo J.*, **6**: 115-119.
21. Lifshitz, B., Fainstein, E., Marcelle, C., Shtivelman, E., Amson, R., Gale, R.P. and Canaani, E. (1988). *Oncogene*, **2**: 113-117.
22. Heisterkamp, N., Knoppel, E. and Groffen, J. (1988). *Nucleic Acids Res.*, **16**: 10069-10081.
23. Rubin, C.M., Blazar, B.R., Hooberman, A.L., Dickler, M.N., Miller, B.A. and Westbrook, C.A. (1988). *Nucleic Acids Res.*, **16**: 8741
24. van der Feltz, M.J.M., Shivji, M.K.K., Grosveld, G. and Wiedemann, L.M. (1988). *Oncogene*, **3**: 215-219.
25. Goodbourn, S.E.Y. Higgs, D.R., Clegg, J.B. and Weatherall, D.J. (1983). *Proc. Natl. Acad. Sci. USA*, **80**: 5022-5026.
26. Proudfoot, N.J., Gil, A. and Maniatis, T. (1982). *Cell*, **31**: 553-563.
27. Jarman, A.P., Nicholls, R.D., Weatherall, D.J., Clegg, J.B. and Higgs, D.R. (1986). *EMBO J.*, **5**: 1857-1863.
28. Bell G. I., Selby, M.J. and Rutter, W.J. (1982). *Nature*, **295**: 31-35.
29. Capon, D.J., Chen, E.Y., Levinson, A.D., Seeburg, P.H. and Goedell, D.V. (1983). *Nature*, **302**: 33-37.
30. Jeffreys, A.J., Wilson, V. and Thein, S.L. (1985). *Nature*, **314**: 67-73.
31. Stratton, R.F., Dobyns, W.B., Airhart, S.D., and Ledbetter, D.H. (1984). *Hum. Genet.*, **67**: 193-200.
32. Schwartz, C.E., Johnson, J.P., Holycross, B., Mandeville, T.M., Sears, T.S., Graul, E.A., Carey, J.C., Schroer, R.J., Phelan, M.C., Szollar, J., Flannery, D.B., Stevenson, R.E. (1988). *Am J. Hum Genet.*, **43**: 597-604.
33. van Tuinen, P., Dobyns, W.B., Rich, D.C., Summers, K.M., Robinson, T.J., Nakamura, Y., Ledbetter, D.H. (1988). *Am. J. Hum. Genet.* **43**: 587-596.
34. Fearon, E.R., Hamilton, S.R. and Vogelstein, B. (1987). *Science*, **238**: 193-196.
35. Heim, S., Billstrom, R., Kristofferson, U., Mandahl, N., Strombeck, B., Mitelman, F. (1985). *Cancer Genet. Cytogenet.* **18**: 215-227.
36. De Braekeleer, M. (1986). *Cancer Genet. Cytogenet.* **23**: 167-170.
37. Huret, JL, Tanzer, J., Henry-Amar, M. (1986). *Hum. Genet.*, **74**: 447-448.
38. McBride, O.W. Merry, D. and Givol, D. (1986) *Proc. Natl. Acad. Sci.*, **83**: 130-134.
39. Vantuinen, P., Dobyns, W.B., Rich, D.C., Summers, K.M., Robinson, T.J., Nakamura, Y. and Ledbetter, D.H. (1988). *Am. J. Hum. Genet.*, **43**: 587-596.