Localization of the c-*abl* oncogene adjacent to a translocation break point in chronic myelocytic leukaemia

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The human c-abl *oncogene maps within the region (q34-qter) of chromosome 9 which is translocated to chromosome 22, the Philadelphia (Ph') chromosome, in chronic myelocytic leukaemia (CML). The position of the Ph' chromosomal break point is shown to be variable and, in one CML patient, has been localized immediately 5' of, or within, the* c-abl *oncogene. A DNA restriction fragment corresponding to this site has been molecularly cloned and shown to represent a chimaeric fragment of DNA from chromosomes 9 and 22.*

HUMAN cellular sequences designated oncogenes have been identified using retroviral transformation-specific sequences as molecular probes. Evidence for the potential involvement of such oncogenes in human cancer is accumulating rapidly. For instance, human tumour-derived DNA sequences, isolated on the basis of their ability to transform mouse cells phenotypically in tissue culture, have been shown to correspond to particular classes of cellular oncogenes[1-5]. More recently, amplification of specific cellular oncogenes in tumour cells has been demonstrated[6-8] and other human oncogenes have been shown to be involved in highly specific chromosomal translocations characteristic of particular classes of human neoplasia[9-13].

The Abelson strain of murine leukaemia virus (MuLV) represents a recombinant between Moloney MuLV and c-*abl*, a cellular oncogene of mouse origin[14]. In contrast to other oncogenic retroviruses, Abelson MuLV transforms lymphoid cells *in vitro*[15] and induces a rapid B-cell lymphoma *in vivo*[16]. We have previously reported the molecular cloning of the v-*abl* homologue (c-*abl*) from a human cosmid library[17]. Human v-*abl* homologous sequences were shown to be dispersed over a total region of around 32 kilobases (kb) and to contain a minimum of six introns. Extensive homology in nucleic acid and amino acid sequence was demonstrated between the tyrosine phosphorylation region of c-*abl* and corresponding regions of the v-*src*, v-*yes* and v-*fes*/*fps* family of viral oncogenes, as well as a more distant relatedness to the catalytic chain of the mammalian cyclic AMP-dependent protein kinase[18]. These findings suggest that each of these oncogenes was probably derived from a common progenitor and may represent members of a diverse, but highly related, family of cellular genes encoding protein kinases.

The human c-*abl* oncogene was initially mapped to the long (q) arm of chromosome 9 (ref. 19). This observation was of interest in view of the frequent involvement of chromosome 9 in the translocation with chromosome 22, t(9; 22), characteristic of chronic myelocytic leukaemia (CML)[20]. Analysis of a series of rodent–human somatic cell hybrids containing either the 9q+ or the 22q− (Philadelphia, Ph') chromosome demonstrated the translocation of human c-*abl* from chromosome 9 to the Ph' chromosome[9]. We show here that in one Ph'-positive CML patient, the chromosomal break point maps within 14 kb 5' of the human c-*abl* oncogene. Such a break point could not be identified at this position in the DNAs of two other patients, arguing that the chromosomal break in Ph'-positive CML can occur at variable sites on chromosome 9. These findings strongly implicate the human c-*abl* oncogene in Ph'-positive CML.

Search for the t(9; 22) junction site

To investigate the possibility that the chromosome 9 break point in the t(9; 22) may be localized either within or in close proximity to the human c-*abl* oncogene, high molecular weight DNA was isolated from biopsy samples of the spleens of three CML patients, each of which contained high percentages of leukaemic cells. In addition, DNA was isolated from an erythroleukaemic cell line, K562, established from a CML patient in blast crisis[21]. Each DNA was digested with *Bam*HI and subjected to Southern blot analysis. As shown in Fig. 1*a*, the restriction enzyme patterns of K562 DNA and DNA of patient 0319129 were indistinguishable from those of control DNA, when hybridized to a previously described[17] v-*abl* probe, $P_{1.7}$. Similarly, no differences in restriction enzyme patterns could be detected in the other two CML DNAs, or in any of the DNAs using other restriction enzymes or probes isolated from different regions of the human c-*abl* locus (not shown).

Chromosomal walking

As no rearrangements could be detected in the v-*abl* homologous cellular DNA region encompassed by our cosmid clones, a probe was prepared corresponding to an 0.4-kb *Hin*dIII–*Eco*RI fragment (0.4 HE) mapping within the 5' domain of the human c-*abl* locus (Fig. 2) and was used to screen a previously described human cosmid library[22]. Although recombinants representative of approximately 10 times the complete human genome were analysed, no positive clones were obtained. Analysis of total human DNA revealed that a *Bam*HI fragment of ~14.5 kb hybridized to the 0.4 HE probe. To characterize this restriction fragment further, a library of size-fractionated *Bam*HI-digested total human DNA was constructed in Charon 30. Screening of the library with the 0.4 HE probe revealed seven positive recombinant phage clones. All positive plaques were extremely small and turbid. The insert of one of these positives was subcloned into pBR328 (p5'-c-*abl*) and subjected to detailed restriction enzyme analysis as shown in Fig. 2*b*. No sequences in the 14.5-kb fragment hybridized to any of a series of previously described[17] probes corresponding to the complete v-*abl* genome.

As indicated in Fig. 2*b*, an 0.2-kb probe was prepared from the 5' terminus of p5'-c-*abl* by digestion with *Bam*HI and *Eco*RI. This probe hybridized to a *Hin*dIII fragment, of ~9.5 kb, in all DNAs examined, suggesting that sequences immediately 5' to the newly cloned region are non-rearranged (Fig. 1*c*, lanes 3–7). As expected, a fragment of 14.5 kb was

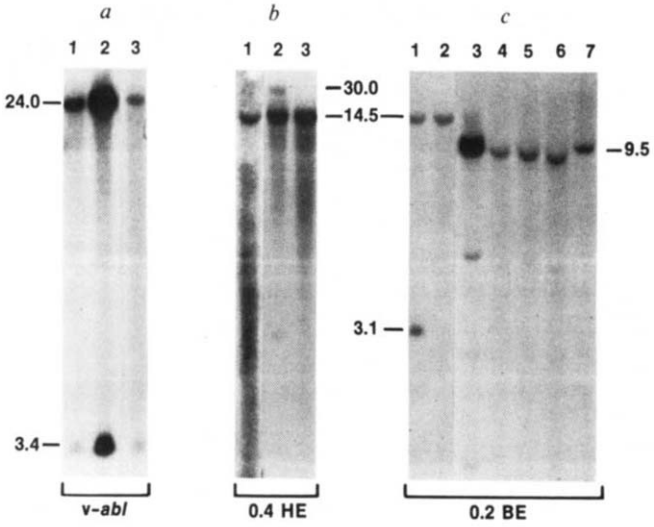

Fig. 1 Identification of DNA sequences mapping within and 5′ of the human c-*abl* locus. *a*, *Bam*HI digest of 10 μg of DNA from human cell line A673 (lane 1), K562 (lane 2) and patient 0319129 (lane 3). *b*, *Bam*HI digest of 10 μg DNA from CML 02120185 (lane 1), CML 0319129 (lane 2) and CML 0311068 (lane 3). *c*, *Bam*HI digest of DNA from patient 0319129 (lane 1), 0311068 (lane 2), *Hin*dIII digest of DNA from cell line K562 (lane 3), CML 02120185 (lane 4), CML 0319129 (lane 5), CML 0311068 (lane 6) and cell line A204 (lane 7). Molecular probes including a previously described v-*abl* probe (v-*abl* $P_{1.7}$)[17], 0.4 HE and 0.2 BE (see Fig. 2) are indicated at the bottom of the figure. Frozen spleen tissue was obtained through the NIH Resources and Logistics Branch, DCCP. Patient 0319129 was a 26-yr-old male, patient 0218105 was a 57-yr-old male and patient 0311068 was a 67-yr-old female. Cell line K562, pedigree 722, (GM 5372) was obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey. DNA was prepared from tissue and cell lines according to Jeffreys and Flavell[30]. DNA was digested with restriction enzymes, electrophoresed on 0.75% agarose gels and blotted according to the method of Southern[31]. Isolation of probes, hybridization and washings, to 0.3×SSC at 65 °C, were as described elsewhere[17,32]. Molecular weights of the hybridizing fragments (kb) are as indicated.

detected in *Bam*HI-digested normal human DNA, while in DNA of CML patient 0319129, an additional *Bam*HI restriction fragment of ~3.1 kb (Fig. 1*c*, lane 1) was identified. Similarly, the 0.4-kb HE probe isolated from the 5′ region of c-*abl* hybridized with a 14.5-kb *Bam*HI fragment in the other two CML DNAs (Fig. 1*b*). In DNA from CML patient 0319129, however, an extra *Bam*HI fragment of ~30 kb was visible (lane 2). The most likely explanation for these findings is that one allelic copy of DNA sequences 5′ to c-*abl* in CML patient 0319129 DNA is normal while the second is rearranged; these appear to be present in about a 1:1 molar ratio.

To define more accurately the region where DNA of patient 0319129 differs from the normal region, probes including an 0.52-kb *Eco*RI fragment (0.52 E) and an 0.76-kb *Eco*RI–*Hin*dIII fragment (0.76 HE) (see Fig. 2*b*) were prepared from p5′-c-*abl*, corresponding to the approximate region of rearranged sequences in the DNA of patient 0319129. As shown in Fig. 3, extra DNA fragments were found with these probes on restriction enzyme digestion of DNA 0319129 with *Bgl*II, *Xba*I, *Sst*I and *Hin*dIII. The 9.5-kb 5′ *Hin*dIII fragment, which hybridized to the 0.52-kb *Eco*RI probe, seemed to be present in a normal quantative level, as did the 2.0-kb fragment hybridizing to the 0.76-kb HE probe. A rearrangement thus appeared to have taken place within the 1.25-kb *Hin*dIII fragment since, in addition to the normal 1.25-kb *Hin*dIII fragment, a fragment of 2.2-kb can be identified.

Molecular cloning

As human c-*abl* is translocated to the Philadelphia chromosome ($22q^-$) in CML, we reasoned that the transposed sequences in DNA of CML patient 0319129 should include most of the 14.5-kb *Bam*HI fragment; the 5′ sequences of this fragment would presumably remain on chromosome 9, linked to sequences from chromosome 22, thus accounting for the 3.1-kb *Bam*HI fragment (Fig. 1*c*, lane 1). The 9.5-kb *Bgl*II fragment (Fig. 3*b*) would thus represent the chromosome 22/9 chimaeric fragment localized at the Ph′ translocation break point; the 6.0-kb *Bgl*II fragment (Fig. 3*b*) would contain sequences from chromosomes 9 and 22, on the 9q+ chromosome. Alternatively, the differences between the DNA of patient 0319129 and control DNA could be the result of DNA rearrangements, deletions or insertions having no connection with the t(9; 22) Ph′ translocation.

To distinguish between these possibilities, DNA from CML patient 0319129 was digested with *Bgl*II, size fractionated in

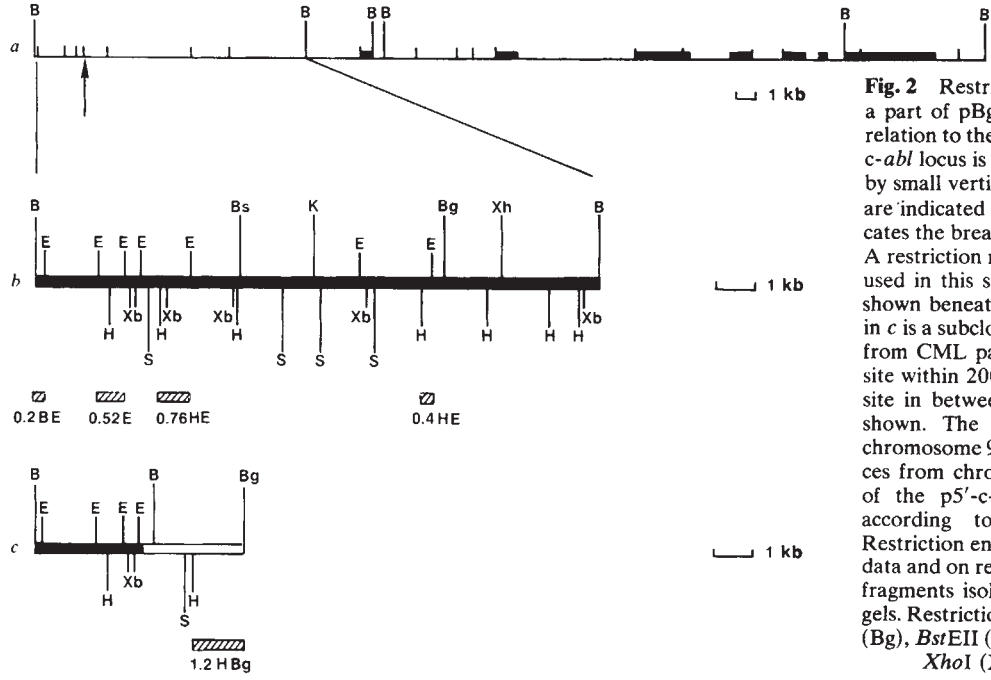

Fig. 2 Restriction enzyme analysis of p5′-c-*abl* and a part of pBglII 9q+. The position of p5′-c-*abl* in relation to the v-*abl* hybridizing region in the human c-*abl* locus is indicated in *a*. *Eco*RI sites are marked by small vertical lines; v-*abl* homologous sequences are indicated by solid bars. The vertical arrow indicates the break point in the DNA of CML 0319129. A restriction map of p5′-c-*abl* is shown in *b*; probes used in this study, indicated as hatched boxes, are shown beneath the map. The pBglII 9q+ fragment in *c* is a subclone of a 6.0-kb *Bgl*II fragment isolated from CML patient 0319129; in *b* and *c* the *Bgl*II site within 200 bp of the 5′ *Bam*HI site and a *Hpa*I site in between these two restriction sites are not shown. The solid bar indicates sequences from chromosome 9 while the open bar represents sequences from chromosome 22. Cloning and subcloning of the p5′-c-*abl* and *Bgl*II-9q+ sequences was according to previously described methods[33]. Restriction enzyme maps are based on hybridization data and on results of double digestions of individual fragments isolated from low melting point agarose gels. Restriction enzymes include: *Bam*HI (B), *Bgl*II (Bg), *Bst*EII (Bs), *Hin*dIII (H), *Sst*I (S), *Xba*I (Xb), *Xho*I (Xh), *Eco*RI (E) and *Kpn*I (K).

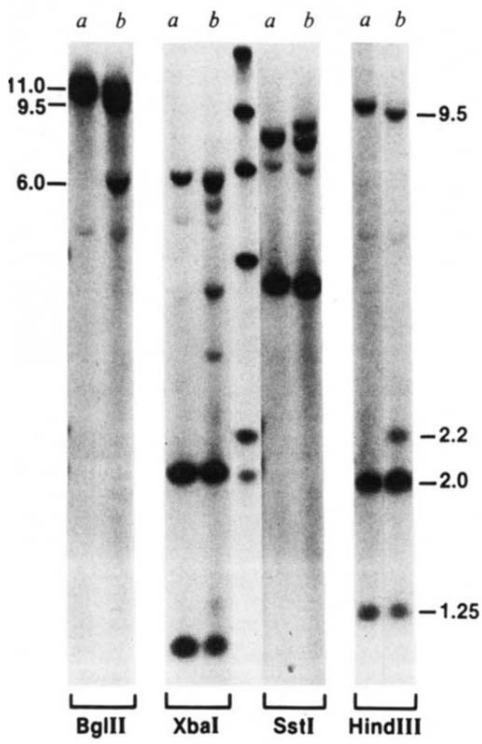

Fig. 3 Restriction enzyme analysis of the DNA of CML patient 0319129. Restriction enzymes used and the molecular weights of the fragments in the *Bgl*II and *Hin*dIII digestions are as indicated. ^{32}P-labelled *Hin*dIII digestion fragments of λ DNA in the centre of the figure serve as a molecular weight marker for the *Xba*I and *Sst*I digestions. Southern blots were hybridized with a mixture of the 0.52 E and 0.76 HE probes (see Fig. 2*b*), as described in Fig. 1 legend. DNA of the cell line A673 is shown in lanes *a*; DNA of CML 0319129 is in lanes *b*.

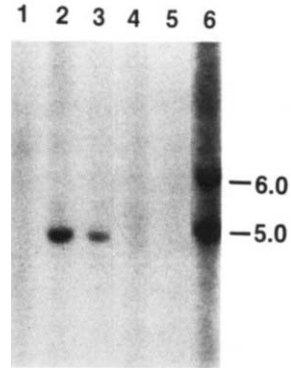

Fig. 4 Chromosomal localization of the 3′ domain of pBglII-9q+. High molecular weight DNAs were digested with *Bgl*II, electrophoresed on an 0.75% agarose gel, blotted and hybridized to the 1.2 HBg probe (see Fig. 2*c*) and included mouse (lane 1), A204 (lane 2), PgMe-25Nu (chromosome 22, lane 3), 10CB-23B (chromosome 9, lane 4), WESP-2A (chromosome 22q-, lane 5) and CML patient 0319129 (lane 6).

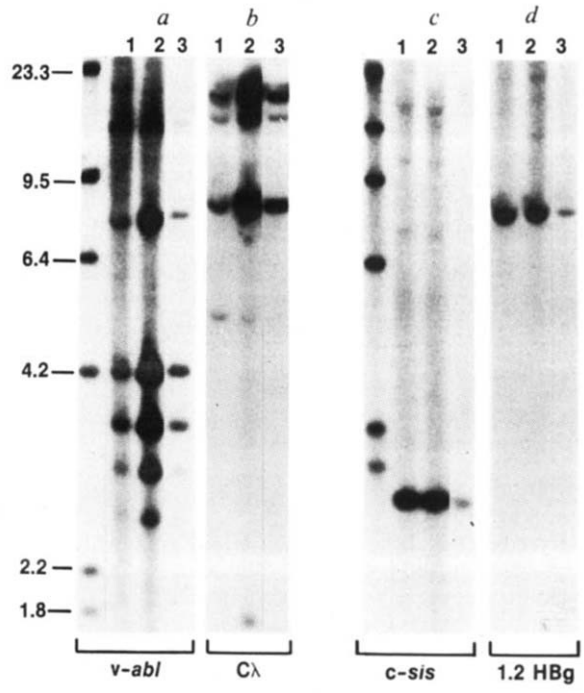

Fig. 5 Amplification of c-*abl* in the erythroleukaemic cell line K562. Control human DNA (10 μg, lanes 1 in each panel) was run next to 10 μg (lanes 2) and 2.5 μg (lanes 3) K562 DNA. ^{32}P-labelled *Hin*dIII-digested λ DNA is included as a molecular weight standard for *a* and *b* (0.6% agarose gels) and for *c* and *d* (0.75% agarose gels). *a*, DNAs were digested with *Eco*RI and hybridized to a previously described v-*abl* probe, v-*abl* $P_{1.7}$ (ref. 17). *b*, *Eco*RI-digested DNAs were hybridized to a *Bgl*II–*Hin*dIII probe, isolated from a human C_λ clone Huλ5 (ref. 25). *c*, *Bam*HI-digested DNA hybridized to a previously described[26] 1.7-kb *Bam*HI probe prepared from a human c-*sis* cosmid clone. *d*, *Bgl*II-digested DNAs hybridized to the 1.2 HBg probe (Fig. 2*c*).

the 6–7-kb range, and a library constructed in *Bam*HI-digested Charon 30 phage arms. Three positive clones were identified on screening with the 0.2-kb BE probe. Because each of the positive clones contained an additional inserted *Bgl*II fragment, some *Bgl*II sites had remained intact, allowing the subcloning of most of the 6.0-kb *Bgl*II fragment by *Bgl*II–*Hpa*I digestion into plasmid ORF[23]. Colonies containing this recombinant plasmid were visible only on a two- to threefold longer incubation at 37 °C than neighbouring non-positive colonies. This particular DNA region may thus contain sequences that inhibit bacterial growth, possibly explaining our failure to detect these sequences in cosmid libraries. As shown in the restriction enzyme map of this fragment (Fig. 2*c*), the *Eco*RI site 3′ of the 0.52-kb *Eco*RI fragment is still present. In contrast, an *Sst*I site immediately 3′ of it and all other restriction enzyme sites to the 3′ are either missing or different from those found in the same region in p5′-c-*abl*. These findings localize a putative break point in p5′-c-*abl* between the *Eco*RI and *Sst*I sites. Sequences 5′ of this point in the 6.0-kb *Bgl*II fragment hybridize to p5′-c-*abl*; sequences 3′ of it do not (results not shown).

To determine the origin of the 6.0-kb *Bgl*II fragment, a 1.2-kb *Hin*dIII–*Bgl*II probe (1.2 HBg) was prepared (Fig. 2*c*). As shown in Fig. 4, using stringent washing conditions, this probe hybridized to a 5.0-kb *Bgl*II fragment in DNA of the human cell line A204 (lane 2), but not to sequences in mouse DNA (lane 1) or in rodent–human somatic cell hybrids containing human chromosome 9 (lane 4)[9]. In an independent hybrid (PgMe-25Nu), however, containing chromosome 22 as its only human component[9], a 5.0-kb *Bgl*II fragment was detected (lane 3). Moreover, using the same probe both 5.0-kb and 6.0-kb *Bgl*II fragments were identified in the DNA of patient 0319129 (lane 6). The 1.2 HBg probe did not hybridize to sequences in the human–mouse somatic cell hybrid WESP-2A (Fig. 4, lane 5), which contains the Philadelphia chromosome in the absence of 9,9q+ or 22. Thus, the 6.0-kb *Bgl*II fragment cloned from patient 0319129 DNA must contain, in addition to sequences from chromosome 9, sequences originating from the translocated region of chromosome 22.

Amplification of c-*abl*

Although the K562 cell line lacks a cytogenetically identifiable Ph′ chromosome (ref. 24 and A. Hagemeijer, personal communication), it was reported to contain a small ring chromosome r(22), which is probably derived from a Ph′ chromosome[24]. Our results suggest that, at a minimum, K562 contains a chromosome(s) resembling the Ph′ chromosome marker. As shown in Fig. 5*d*, using the 1.2 HBg probe, a non-amplified 5.0-kb *Bgl*II fragment was detected in K562 DNA. In contrast, human c-*abl* sequences are amplified at least fourfold in K562 DNA, as are

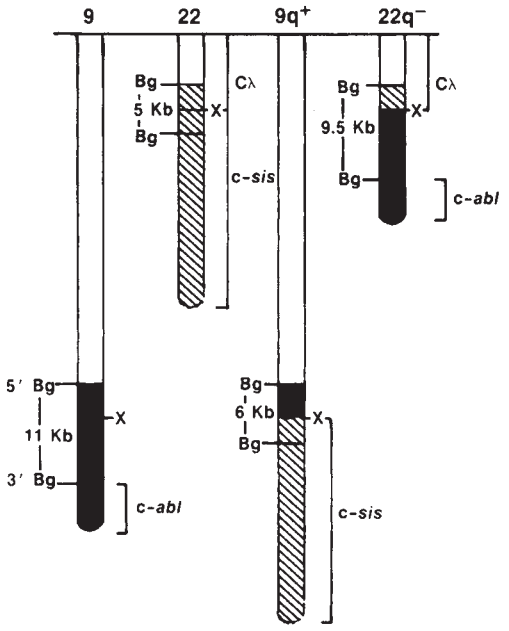

Fig. 6 Schematic representation of the t(9; 22) in CML patient 0319129. The sizes of the *Bgl*II fragments detected with probes from chromosome 9 and 22 are indicated. Regions in black originate from chromosome 9; hatched regions are from chromosome 22. An *X* indicates the t(9; 22) chromosomal break point. The human c-*sis* oncogene and the C_λ region on chromosome 22 are shown for purposes of orientation; human c-*sis* is localized on chromosome 22 (ref. 34) and is translocated to chromosome 9 in CML[26]. C_λ is localized in band q 11 of chromosome 22 (ref. 10), and is not involved in the Ph′ translocation (unpublished results).

sequences up to 17 kb upstream of the most 5′ v-*abl* homologous *Eco*RI region (Fig. 1*c*). A 11.0-kb *Eco*RI fragment (Fig. 5*a*) that hybridizes to the v-*abl* $P_{1.7}$ probe but has no linkage with the main human c-*abl* locus[17] is non-amplified and thus serves as an internal control. The immunoglobulin light-chain constant region (C_λ)[25] is also amplified at least fourfold (Fig. 5*b*). This latter observation is consistent with the localization of human c-*abl* and C_λ sequences on the same amplification unit, presumably a part of the Ph′ chromosome. In concordance with this conclusion, C_λ remains on the Ph′ chromosome in the t9:22 (unpublished observations). In contrast, another human oncogene, c-*sis*, which is located on chromosome 22 and transferred to chromosome 9 in the Ph′ translocation[26], is non-amplified (Fig. 5*c*). Finally, the fact that the 5.0-kb *Bgl*II fragment normally localized on chromosome 22, is non-amplified in K562, supports the assumption that it is translocated to chromosome 9 in this cell line.

Conclusions

The present findings establish that in one of three CML patient DNAs examined, the Ph′ chromosomal break point is localized within 14 kb of the most 5′ v-*abl* homologous region. Because the position of the most 5′ exon in human c-*abl* is not known, the possibility that the t(9; 22) break point may even map within a coding region of the human c-*abl* locus cannot be excluded. We have not localized the break points in the DNAs of the two other CML patients or in DNA of the cell line WESP-2A, containing the 22q− chromosome. Our findings, however, establish that the site of the Ph′ translocation break point is variable and suggest that break points in the latter DNAs may be located 5′ of the ~80 kb of cloned DNA encompassing the human c-*abl* locus. Similarly, the t(8;14) translocation in Burkitt lymphoma, involving the human c-*myc* oncogene, does not appear to involve a single site[10–12,27]. The orientation of human c-*abl* on chromosome 9, with its 5′ region towards the centromere of the chromosome (Fig. 6), is established by the fact that the break point has been found 5′ to the human c-*abl* locus.

K562 cells lack a cytogenetically identifiable Ph′ chromosome despite their derivation from a CML patient in blast cell crisis[21]. However, genes on either side of the break point in the Ph′ chromosome, c-*abl* and C_λ, are not only present in this cell line, but are amplified. In contrast, no amplification was found in the case of c-*sis*, normally on chromosome 22 but translocated to chromosome 9 in Ph′-positive CML, or a DNA region translocated to chromosome 9 corresponding to a restriction fragment containing the t(9; 22) break point isolated from a CML patient. The amplification seems to be specific; neither human c-*fes*, on chromosome 15 (ref. 19), nor c-*fms*, on chromosome 5 (ref. 28), is amplified (data not shown). The finding that c-*abl* is amplified in K562 can be explained in two ways: it can reflect the fact that these cells were grown in tissue culture, possibly resulting in specific chromosomal aberrations and/or amplifications; alternatively, the c-*abl* may have been amplified during the blast crisis of the patient, the overproduction of the c-*abl* gene product being the cause of the disease. The relative amplification of a region of DNA, which includes both c-*abl* and C_λ sequences, suggests that the two are nearly contiguous in this cell line.

We have previously demonstrated the translocation of a small piece of chromosome 9 to chromosome 22 in CML patients carrying the Ph′ chromosome[9]. The size of this fragment was calculated to be less than 5,000 kb, suggesting a potential role of the c-*abl* oncogene in CML. The isolation, in the present study, of a region of DNA from a CML patient that contains a chromosomal break point which is localized within 14 kb of the c-*abl* locus, the specific amplification of c-*abl* sequence in the K562 cell line, and the finding that c-*abl* is translocated to chromosome 22 in complex Ph′ translocations[29] further implicate the c-*abl* oncogene in chronic myelocytic leukaemia.

We thank A. Geurts van Kessel for providing somatic cell lines, A. Hagemeijer for valuable discussion, P. Leder for the generous gift of the recombinant Huλ5, Gail Blennerhassett for technical assistance and Beverly Bales for help with the preparation of the manuscript. The work was supported by the Netherlands Cancer Society (Koningin Wilhelmina Fonds) and under NCI PHS contract NOI-CP-75380. C.R.B. is a recipient of a fellowship from the Deutsche Forschungsgemeinschaft.

Received 8 July; accepted 2 September 1983.

1. Pulciani, S. *et al. Nature* **300,** 539–542 (1982).
2. Der, C. J., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79,** 3637–3640 (1982).
3. Tabin, C. J. *et al. Nature* **300,** 143–149 (1982).
4. Taparowsky, E. *et al. Nature* **300,** 762–765 (1982).
5. Shimizu, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 2112–2116 (1983).
6. Collins, S. & Groudine, M. *Nature* **298,** 679–681 (1982).
7. Dalla-Favera, R., Wong-Staal, F. & Gallo, R. *Nature* **299,** 61–63 (1982).
8. Alitalo, K., Schwab, M., Lin, C., Varmus, H. E. & Bishop, J. M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1707–1711 (1983).
9. de Klein, A. *et al. Nature* **300,** 765–767 (1982).
10. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7837–7841 (1982).
11. Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M. & Cary, S. *Proc. natn. Acad. Sci. U.S.A.* **80,** 1982–1986 (1983).
12. Dalla-Favera, R. *et al. Science* **219,** 963–967 (1983).
13. Sheer, D. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 5007–5011 (1983).
14. Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. *Cell* **22,** 777–785 (1980).
15. Rosenberg, N., Baltimore, D. & Scher, C. *Proc. natn. Acad. Sci. U.S.A.* **72,** 1932–1936 (1975).
16. Abelson, H. T. & Rabstein, L. S. *Cancer Res.* **30,** 2213–2222 (1970).
17. Heisterkamp, N., Groffen, J. & Stephenson, J. R. *J. molec. appl. Genet.* **2,** 57–69 (1983).
18. Groffen, J., Heisterkamp, N., Reynolds, F. Jr. & Stephenson, J. R. *Nature* **304,** 167–169 (1983).
19. Heisterkamp, N. *et al. Nature* **299,** 747–750 (1982).
20. Rowley, J. D. *Nature* **243,** 290–293 (1973).
21. Lozzio, C. B. & Lozzio, B. B. *Blood* **45,** 321–334 (1975).
22. Groffen, J., Heisterkamp, N., Grosveld, G., van de Ven, W. & Stephenson, J. R. *Science* **216,** 1136–1138 (1982).
23. Weinstock, G. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **80,** 4432–4436 (1983).
24. Klein, E. *et al. Int. J. Cancer* **18,** 421–431 (1976).
25. Hieter, P. A., Hollis, G. F., Korsmeyer, S. J., Waldmann, T. A. & Leder, P. *Nature* **294,** 536–540 (1981).
26. Groffen, J. *et al. J. exp. Med.* **158,** 9–15 (1983).
27. Stanton, L. W., Watt, R. & Marcu, K. B. *Nature* **303,** 401–406 (1983).
28. Groffen, J. *et al. Nucleic Acids Res.* (in the press).
29. Bartram, C. R. *et al. Nature* **306,** 277–280 (1983).
30. Jeffreys, A. J. & Flavell, R. A. *Cell* **12,** 429–439 (1977).
31. Southern, E. M. *J. molec. Biol.* **98,** 503–517 (1975).
32. Bernards, R. & Flavell, R. A. *Nucleic Acids Res.* **8,** 1421–1533 (1980).
33. Groffen, J., Heisterkamp, N., Blennerhassett, G. & Stephenson, J. R. *Virology* **126,** 213–227 (1983).
34. Swan, D. C. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 4691–4696 (1982).