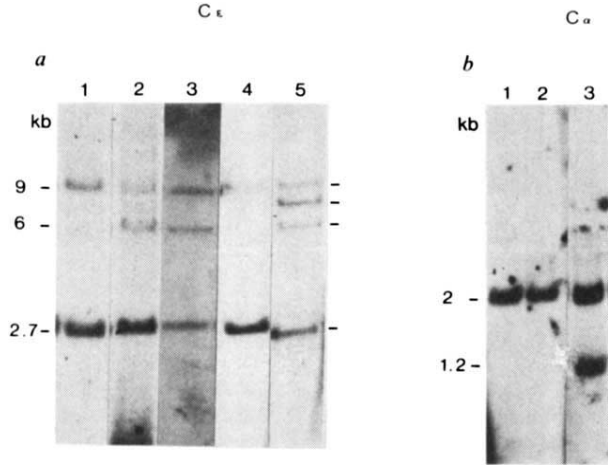

Fig. 3 Southern blot hybridization of Cε and Cα probes to genomic DNA digested with *Bam*HI (*a*) or *Pst*I (*b*). The DNA digests were: *a*, Probe: ε; slot 1, TAK3; slot 2, TAK14; slot 3, LAT; slot 4, TOU; slot 5, BOU. *b* Probe: α; slot 1, TOU; slot 2, TAK3; slot 3, TAK92.
Methods: Complete digests were separated on 0.8% agarose (*a*) or 1.4% agarose (*b*), blotted and hybridized (as in Fig. 1) with Cε probe (*a*) or Cα probe (*b*). The size of fragments (given on the left of each panel) were estimated relative to λ phage DNA cut with *Hin*dIII. The lines on the right of *a* mark the positions of fragments discussed in the text.

homozygosity.increases and such individuals are readily detected during random immunoglobulin haplotype screening.

Finally, the consistent deletion pattern of C_H genes in TAK, TOU and EZZ allows a link between the absence of C_H genes and the overall ordering of the human C_H genes described in the accompanying paper[10]. Two groups of cosmid clones have been described which seem to encompass γ3–γ1–ψε1–α1 (region A) and γ2–γ4–ε–α2 (region B)[10]. As it seems that the deletions described here include γ1, γ2, γ4, ψε1 and α1 genes, the most probable order for the groups of cosmid clones is 5′ region A–region B 3′ as the deletions presumably start downstream of γ3 (region A) and end upstream of the active ε gene (region B). We are now using gene cloning studies to clarify these issues as well as the nature and localization of the points of deletion within the C_H genes.

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Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change

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Several different transforming genes have been observed in the DNA of a variety of tumours and tumour cell lines of human and rodent origin by the ability of these genes to induce morphological transformation in NIH 3T3 cells[1–5]. The transforming gene found in a human bladder carcinoma cell line, T24, is H-*ras*-1, the human homologue of the Harvey sarcoma virus oncogene (v-H-*ras*)[6–9]. In the present study we have compared the H-*ras*-1 genes cloned from T24 and normal human DNA. The H-*ras*-1 gene cloned from T24 DNA induces transformation in NIH 3T3 cells, while the same gene cloned from normal cellular DNA does not. The functionally significant difference between the transforming and normal genes appears to be a single base mutation, which produces an amino acid change in the sequence of the proteins that the genes encode.

We had previously isolated transforming sequences from NIH 3T3 cells that had been transformed with DNA from T24 bladder carcinoma cells[10]. These sequences had undergone some rearrangement during gene transfer into NIH 3T3 cells. As a result of passage in NIH 3T3 cells, the transforming gene might have undergone less readily detectable secondary changes altering its biological activity. Therefore, in order to properly compare the transforming sequences of T24 to their normal counterparts, we directly cloned the H-*ras*-1 sequences from λ libraries constructed from T24 DNA and human placental DNA. From the T24 library we obtained seven independent isolates, all of which contained a 6.2-kilobase (kb) *Bam*HI H-*ras*-1 fragment with an identical restriction endonuclease pattern. From the placental library, we obtained clones of two types: in three (P3 type), H-*ras*-1 sequences were carried on a 6.7-kb *Bam*HI fragment: in the four others (P1 type), H-*ras*-1 sequences were carried on a 8.3-kb *Bam*HI fragment (see Fig. 1). Differences in the size of the *Bam*HI fragments were expected due to restriction endonuclease fragment length polymorphism about this gene in the human population[10].

We tested all of our isolates for the ability to induce foci of morphologically transformed NIH 3T3 cells on DNA mediated gene transfer. DNA from all T24 isolates of H-*ras*-1 efficiently transformed NIH 3T3 cells, while DNA from all placental isolates failed to induce foci. This difference must be the result of differences in DNA sequence. To facilitate our search for these differences, we tested the transforming activity of chimaeric genes. The restriction endonuclease *Xba*I cleaves the *Bam*HI fragment containing the H-*ras*-1 gene into one small and one large fragment (see Fig. 1). The large and small fragments were purified from each of the three cloned genes (types T24, P1 and P3), mixed and ligated in various combinations and finally cleaved with *Bam*HI. All (and only) molecules containing the small T24 *Bam*HI/*Xba*I fragment were capable of inducing transformed foci in NIH 3T3 cells (see Table 1). To further limit the area of our search we used restriction endonuclease *Mst*II, which cleaves the small *Bam*HI/*Xba*I fragment once. The 1.7-kb *Bam*HI/*Mst*II fragment and the 0.24-kb *Mst*II/*Xba*I fragment from the T24 or P3 H-*ras*-1 genes, and the large *Xba*I/*Bam*HI fragment from the P3 gene were purified, mixed and ligated in various combinations, and then cleaved with *Bam*HI. All molecules containing the 0.24-kb *Mst*II/*Xba*I fragment from T24 induced transformed foci in

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NIH 3T3 (see Table 1). The sequence changes in this region are therefore sufficient to confer the transforming capacity on the P3 H-*ras*-1 gene. A few foci are seen in one other combination of fragments (see Table 1) but we hesitate to ascribe significance to this.

We sequenced the 245-bp *Mst* ≤ I*Xba*I fragment in its entirety from P1, P3 and T24 H-*ras*-1 genes. The sequences were identical at all but two nucleotide positions (see Fig. 1). These lie within the first coding exon of the H-*ras*-1 gene as determined by a comparison of genomic sequences to those of an H-*ras*-1 cDNA clone. (O.F. *et al.*, manuscript in preparation).

We found that P1 and P3 H-*ras*-1 genes encoded glycine (GGC) at amino acid 12, while the T24 gene encodes valine (GTC) at that position, the result of a single G→T nucleotide change. We also noted that while all three genes encode histidine at amino acid 27, the codon usage differs (see Fig. 1). Because this latter change does not alter the protein sequence we believe it is not functionally significant (see below).

The above data suggest that an amino acid substitution in the T24 H-*ras*-1 gene product generates a protein with greatly enhanced transforming activity. To test this prediction, we have introduced the T24 and placental H-*ras*-1 genes into NIH 3T3 cells using the pSV2gpt vector of Mulligan and Berg[11]. In this way, we obtained 10 xgprt$^+$ lines containing the T24 H-*ras*-1 gene and 16 lines containing the P3 H-*ras*-1 gene, as determined by Southern filter blot hybridization (see Fig. 2). All ten of the lines containing the T24 H-*ras*-1 gene appeared morphologically transformed, while only two of the lines containing the P3 H-*ras*-1 gene had any of the features we associate with morphological transformation. To measure more precisely the degree of growth transformation we examined the ability of several lines to grow in agar suspension (see Fig. 2). To correlate degree of transformation with gene expression, we measured H-*ras*-1 RNA by filter hybridization and H-*ras*-1 protein by immunoprecipitation of ^{35}S-methionine labelled cell lysates (see Fig. 2). The human H-*ras*-1 gene encodes a 21,000-MW protein (p21) which cross-reacts with antibody to the Harvey v-*ras* p21 (given by M. Furth and E. Scolnick).

In these studies, the only difference we can discern between the expression of the P3 and T24 H-*ras*-1 genes is the potency of their respective protein products. Highly transformed cell lines containing the T24 H-*ras*-1 gene (XT1 and XT3) contain far less *ras* p21 than do minimally transformed lines expressing the normal *ras* gene (XP1, XP3). We estimate from this that the T24-encoded *ras* p21 is at least 100 times more potent at inducing growth transformation than the P3-encoded *ras* p21.

We note that one line (XP2) containing the P3 H-*ras*-1 gene and expressing the highest levels of *ras* p21 does grow in agar to a significant extent. Thus it appears that normal H-*ras*-1 can mediate transformation if the gene product is overproduced. Other workers have reported that a normal allele of H-*ras*-1 acquires transforming activity when physically linked to a high efficiency retroviral promoter[12]. However, very high levels of the normal gene product are required to achieve the degree of

Table 1 Focus induction with chimaeric H-*ras*-1 genes

(5′)*Bam*HI *Xba*I	*Xba*I *Bam*HI(3′)	Foci per 0.1 μg*
T24	T24	358
T24	P1	142
T24	P3	80
P1	T24	0
P1	P1	0
P3	T24	0
P3	P3	0

(5′)*Bam*HI/*Mst*II†	*Mst*II/*Xba*I†	*Xba*I/*Bam*HI(3′)†	Foci per 0.02 μg‡
T24	T24	P3	88
T24	P3	P3	2
P3	T24	P3	163
P3	P3	P3	0

The T24, P1 and P3 *Bam*HI fragment containing the H-*ras*-1 gene was cleaved with *Xba*I and the respective 5′ (*Bam*HI/*Xba*I) and 3′ (*Xba*I/*Bam*HI) halves of the genes (see Fig. 1) purified from agarose gels as previously described[25]. The various 5′ and 3′ halves were mixed in equimolar ratios in the combinations indicated above, ligated with T4 DNA ligase at a DNA concentration of 100 μg ml^{-1}, and then digested with *Bam*HI. The amount of material in the correct molecular form was then estimated by ethidium bromide staining after electrophoresis in agarose gels.

* Approximately 0.1 μg of the chimaeric genes was mixed with 90 μg of NIH 3T3 carrier DNA and added to 3 plates of NIH 3T3 as a calcium phosphate precipitate as previously described[4]. Morphologically altered foci of NIH 3T3 cells were scored after 14 days.

† The 1.7-kb *Bam*HI/*Mst*II and the 0.24-kb *Mst*II/*Xba*I fragments were purified from the T24 and P3 *Bam*HI fragments (see Fig. 1), mixed in equimolar ratio with the 4.8-kb P3 *Xba*I/*Bam*HI fragment, ligated with T4 DNA ligase at a DNA concentration of 100 μg ml^{-1} and then cleaved with *Bam*HI. The amount of material in the correct molecular form was then estimated by ethidium bromide staining after gel electrophoresis.

‡ Approximately 20 ng of the chimaeric genes were mixed with 180 μg of NIH 3T3 carrier DNA and added to six plates of NIH 3T3. Foci were scored after 14 days.

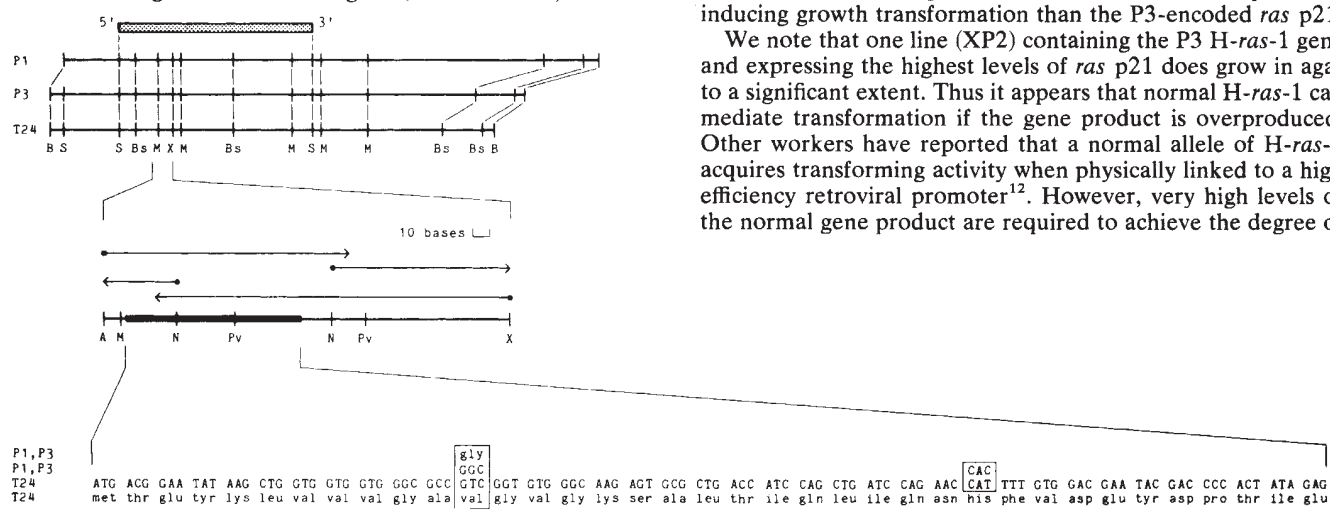

Fig. 1 Structural differences between human H-*ras*-1 genes. The *Bam*HI fragment containing the H-*ras*-1 gene was cloned from placental DNA and T24 DNA libraries constructed in the L47.1 cloning vector[13]. Phage libraries were constructed by the method of Hohn and Murray[14] and screened without prior amplification by the method of Benton and Davis[15]. Restriction maps of the three gene types (P1 and P3 from placenta, and T24 from T24 cells) are shown above. In the region of the 2.9-kb *Sst*I fragment (shaded area) which contains a complete H-*ras*-1 gene (unpublished data) the restriction maps are similar. The 245-bp *Mst*II/*Xba*I fragment, which contains the functionally significant structural differences, was sequenced by the method of Maxam and Gilbert[16] in entirety for all three genes. The sequencing strategy is shown in the middle panel. Restriction fragments were labelled at their 3′ ends with the large fragment of DNA polymerase I[17]. All regions were sequenced at least once on both strands. The bold line indicates the first coding exon of 37 amino acids, determined by sequencing a T24 H-*ras*-1 cDNA clone (O.F. *et al.*, manuscript in preparation). In the bottom panel is shown the DNA sequence of T24 in this coding region and the predicted amino acid sequence. Where P1 and P3 differed from T24 are enclosed in boxes. Restriction endonucleases used were *Ava*I (A), *Bam*HI (B), *Bst*EII (Bs), *Mst*II (M), *Nar*I (N), *Pvu*II (Pv), *Sst*I (S) and *Xba*I (X).

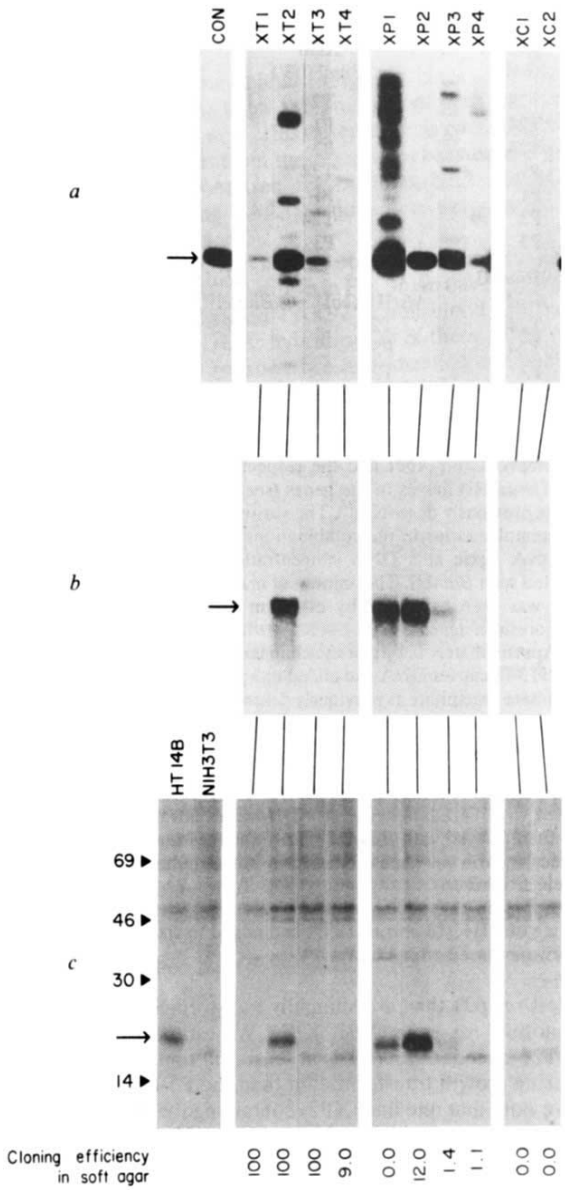

Fig. 2 Expression of normal and transforming H-*ras*-1 genes after co-transfer into NIH 3T3 cells. NIH 3T3 cells containing the human H-*ras*-1 gene cloned from T24 (XT1 . . . XT4) or from placental DNA (XP1 . . . XP4) were obtained by co-transfer[18] after selection for expression of the pSV2gpt vector[11] added in the presence of an excess of the purified *Bam*HI fragments containing the H-*ras*-1 gene. Cells were analysed for H-*ras*-1 gene sequences (*a*), expression of H-*ras*-1 transcripts (*b*), expression of the p21 *ras* gene product (*c*), and cloning efficiency in soft agar (bottom). XC1 and XC2 are NIH 3T3 cells containing the pSV2gpt vector but no human H-*ras*-1 gene. HT14B are NIH 3T3 cells transformed with unintegrated Harvey sarcoma viral DNA. *a*, 6 μg DNA from the indicated cell lines were cut with *Sst*I, electrophoresed in 1.0% agarose gels, filter-blotted according to Southern[19] and filters hybridized with 2.9-kb *Sst*I fragment purified from the human H-*ras*-1 gene (see Fig. 1) and nick translated with ^{32}P (ref. 20) to a specific activity of 3×10^8 cpm μg^{-1}. Filter hybridization and washing conditions were at 72 °C under stringent conditions as previously described[4]. Filters were autoradiographed at −70 °C for 15 h with XR5 film and intensifying screens. Lane labelled CON represents 100 ng of purified 2.9-kb *Sst*I fragment. The presence of the 2.9-kb *Sst*I fragment (arrow) indicates the presence of at least one functional copy of the H-*ras*-1 gene. Other hybridizing fragments represent integration events in which one or both of the *Sst*I sites flanking the H-*ras*-1 gene were destroyed. *b*, Cytoplasmic RNA from the indicated cell line was prepared[21] and 20 μg were electrophoresed in 1.0% agarose formaldehyde gels as previously described[10], filter blotted and hybridized according to Thomas[22] except that dextran sulphate was omitted. The hybridization probe was as described above. The arrow indicates the position of the 1.1–1.2-kb H-*ras* mRNA. *c*, Indicated cells were labelled for 6 h in methionine-free Dulbecco's medium containing 5% dialysed serum and 100 μCi ml^{-1}, 80 Ci $mmol^{-1}$ ^{35}S-methionine. Washed cells were lysed in 1% Triton, 0.5% deoxycholate in phosphate-buffered saline containing 2 μg aprotinin (Sigma) and 1 mM phenylmethylsulphonylfluoride, and centrifuged at 100,000*g* for 12 h. Supernatants were preabsorbed with goat anti-rat IgG and *Staphylococcus aureus* protein A. Immunoprecipitation was performed for 5 h at 4 °C with anti-v-H-*ras* p21 monoclonal antibody, the generous gift of E. Scolnick and M. Furth. After addition of goat anti-rat IgG, immune complexes were absorbed to protein A. Pellets were boiled in SDS sample buffer[23] and analysed by SDS-polyacrylamide gel electrophoresis by the method of Blattler *et al.*[24]. Proteins were visualized after fluorography by exposure for 30 h to Kodak XR5 film. The arrow marks the position of migration of v-H-*ras* p21. Arrowheads are size markers ($MW\times10^{-3}$) from ^{14}C-labelled standards (bovine serum albumin, ovalbumin, carbonic anhydrase and lysozyme; Amersham). Cloning efficiency in soft agar was determined by plating 10^5 cells in 5-cm dishes containing 0.35% Difco agar and Dulbecco's medium with 10% calf serum. 14 days later, numbers of colonies with greater than 10 cells were tabulated for each cell line by examination of six 2-mm diameter microscopic fields.

growth transformation mediated by low levels of an altered *ras* gene product.

We and others have shown transforming *ras* genes are present in many different human tumour cell lines[6–9]. We speculate that the activation of *ras* genes in these other cell lines is the consequence of somatic mutations in coding regions. If such mutations do occur in human tumour cells, the altered protein products might be immunogenic in their human hosts. The presence, therefore, of antibodies against *ras* proteins in humans may be diagnostic for certain forms of cancer.

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Note added in proof: Substantially similar findings were recently reported for the transforming genes of T24 (ref. 26) and EJ (ref. 27), another bladder carcinoma cell line which we believe has arisen from T24 (M.W., M.G., J. Fogh, N. Dracopoli and M. Pollack, unpublished data). Most importantly, the predicted amino acid sequences are all in agreement. We note the following differences in the critical 245-bp *Mst*II/*Xba*I fragment: (1) a *Mst*II cleavage site 9 bases 5′ to the ATG start codon is predicted and present in the genes we have sequenced, but not predicted by the published sequences of the other groups. (2) A *Nar*I cleavage site 104 bases 3′ to the ATG start codon is predicted and present in the genes we have sequenced, but not found in the sequence published by Tabin *et al.*[27]. (3) An altered histidine codon is not observed for the normal gene sequenced by the other groups.

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A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia

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The transforming genes of oncogenic retroviruses are homologous to a group of evolutionary conserved cellular *onc* genes[1]. The human cellular homologue (c-*abl*) of the transforming sequence of Abelson murine leukaemia virus (A-MuLV) was recently shown[2] to be located on chromosome 9. The long arm of this chromosome is involved in a specific translocation with chromosome 22, the Philadelphia translocation (Ph[1]), t(9; 22) (q34, q11), which occurs in patients with chronic myelocytic leukaemia (CML)[3–5]. Here we investigate whether the c-*abl* gene is included in this translocation. Using c-*abl* and v-*abl* hybridization probes on blots of somatic cell hybrids, positive hybridization is found when the 22q$^-$ (the Philadelphia chromosome), and not the 9q$^+$ derivative of the translocation, is present in the cell hybrids. From this we conclude that in CML, c-*abl* sequences are translocated from chromosome 9 to chromosome 22q$^-$. This finding is a direct demonstration of a reciprocal exchange between the two chromosomes[6] and suggests a role for the c-*abl* gene in the generation of CML.

The human c-*abl* sequences represent a cellular homologue of the transforming component of A-MuLV. This retrovirus is a recombinant between Moloney MuLV and mouse cellular c-*abl* sequences[7] and induces lymphoid tumours on *in vivo* inoculation of the mouse[8,9]. The major A-MuLV translational product has been identified as a poly-protein, P120$^{gag-abl}$, consisting of amino-terminal structural proteins encoded by the M-MuLV *gag* gene, linked to an acquired cellular sequence encoded carboxy-terminal component[10,11]. This protein is one of several virus-encoded transforming proteins with tyrosine-specific protein kinase activity[12–15]. Similar oncogenic sequences of Harvey and Kirsten sarcoma virus are homologous to transforming sequences (c-Ha-*ras*, c-Ka-*ras*) isolated from human bladder and lung carcinoma cell lines[16–18]. Both these sequences induce transformation of mouse NIH 3T3 cells after transfection, establishing that the human genes have potential transforming activity. Recently, the human c-*abl* gene has been cloned in cosmids[19]. Using v-*abl* DNA as a probe, several clones containing overlapping sequences representing the entire c-*abl* gene were isolated from a human lung carcinoma cosmid library. The restriction enzyme map of the human v-*abl* cellular homologue, presented in Fig. 1, identifies areas of the gene which hybridize to v-*abl* sequences. The gene is distributed over a region of 40 kilobases (kb) of human DNA and contains multiple intervening sequences. On transfection of Rat-2 cells with the c-*abl* cosmids, no transforming activity was detected, not unexpectedly, as none of the cosmid clones tested contained the entire c-*abl* gene[19].

By Southern blot analysis of a series of somatic cell hybrids, the human c-*abl* gene has been localized on chromosome 9[2]. This finding is of interest because of the involvement of the long arm of chromosome 22 (band 22q11) in a specific translocation with the long arm of chromosome 9 (band 9q34), the Philadelphia translocation (Ph[1]), occurring in human CML[3,4]. The abnormal chromosomes are designated 9q$^+$ and 22q$^-$; of these, the 22q$^-$ chromosome is observed in 92% of CML cases[5]. We investigated the chromosomal location of the human c-*abl*

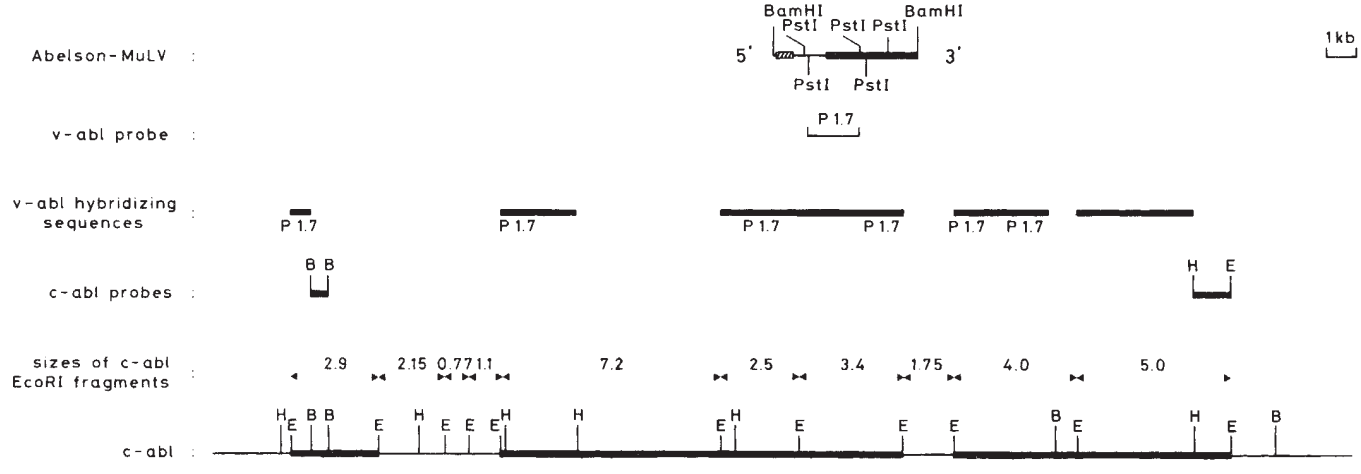

Fig. 1 Restriction enzyme map of the human c-*abl* region[19]. The upper line of the figure shows the *Bam*HI subclone of A-MuLV; the hatched box presents the long terminal repeat, the solid bar the acquired cellular sequences. Directly beneath the A-MuLV genome, a subgenomic *Pst* 1.7-kb fragment, used as a probe in this study, is shown. Human c-*abl* DNA restriction fragments homologous to v-*abl* sequences are indicated as black boxes and those that show homology to the 1.7-kb *Pst* v-*abl* fragments are designated by P 1.7. The third line shows the human c-*abl* 0.6-kb *Bam*HI and 2.2-kb *Hin*dIII–*Eco*RI probes, which hybridize to 5′ and 3′ c-*abl* *Eco*RI fragments, respectively. The sizes of all *Eco*RI c-*abl* fragments are indicated on the fourth line. The bottom line represents the restriction enzyme map of the human c-*abl* gene. Restriction enzymes include *Bam*HI (B), *Hin*dIII (H) and *Eco*RI (E). A more detailed characterization of the human c-*abl* locus will be published elsewhere[19].

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