Biochimica et Biophysica Acta, 989 (1989) 209–224
Elsevier

BBACAN 87210

abl genes

Lalita Ramakrishnan [1] and Naomi Rosenberg [2]

Departments of [1] Pathology, [2] Molecular Biology and Microbiology and [1] Medicine, and [1,2] The Immunology Graduate Program, Tufts University School of Medicine and [1] The New England Medical Center Hospitals, Boston, MA (U.S.A.)

(Received 19 June 1989)
(Revised manuscript received 13 September 1989)

Contents

Abbreviations: CML, chronic myelogenous leukemia; IL, interleukin; *ts*, temperature-sensitive; GAP, GTPase-activating protein; LTR, long terminal repeat; ALL, acute lymphocytic leukemia; MA, p15 protein; CA, p30 protein; *arg*, *abl*-related gene; Mo-MLV, Moloney murine leukemia virus; RFLP, restriction fragment length polymorphism.

Correspondence: N. Rosenberg, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, U.S.A.

I. Historical perspective

The oncogene *abl* was first discovered in Abelson murine leukemia virus (Ab-MLV) [1]. This virus was isolated about 6 years prior to the discovery that genes contained in some retroviruses were homologous to normal cellular genes (reviewed in Ref. 2) and was initially of interest because, unlike most murine leukemia viruses (reviewed in Ref. 3), Ab-MLV induced lymphomas after a very short latent period (reviewed in Ref. 4). The ability of Ab-MLV to transform both fibroblasts [5] and lymphoid cells [6,7] in vitro heightened interest in the virus and emphasized the biological differences that distinguish Ab-MLV from other murine leukemia viruses. However, these early experiments only scratched the surface of the varied and complex responses of cells to *abl* expression.

The identification of the Ab-MLV transforming protein and the finding that many normal cells encode a related protein [8–10] pointed the way for experiments that culminated in the cloning of the viral genome [11] and the normal cellular homologue of the viral transforming gene [12]. These seminal experiments defined the properties of Ab-MLV and set the stage for unraveling the relationship of the virus to the normal cellular *abl* gene. In the 1980s, attention has focused on understanding the mechanism by which Ab-MLV induces transformation, an area that has been the subject of several recent reviews [4,13,14]. A second major area of investigation has centered on characterizing the structure and expression of c-*abl* and elucidating its role in growth, differentiation and malignant processes. This review will focus on recent progress in this latter area.

II. Forms of *abl* protein

II-A. Overview

Even before the sequence of any of the *abl* genes was known, analyses of the *abl* protein encoded by Ab-MLV revealed that the molecule was one of an ever-increasing number of protein tyrosine kinases [15,16]. Expression of a portion of the v-*abl* sequence in *E. coli* confirmed that the kinase activity was an intrinsic property of the v-*abl* protein [17]. The typical increase in cellular phosphotyrosine [18] was shown to be associated with transformation and analyses of viral mutants demonstrated that an active tyrosine kinase was required for the response [19–21]. Later technical refinements revealed that all forms of *abl* protein had tyrosine kinase activity when assayed in vitro [22,23].

Multiple forms of *abl* protein, all named for their molecular weights as estimated by migration in SDS-PAGE, have been described. The viral forms are found in the murine retrovirus, Ab-MLV and the feline retrovirus, HZ-2 feline sarcoma virus. In the case of Ab-MLV, a host of strains has been described but all of these arose from the Ab-MLV-P160 strain [24,25], the original wild-type strain. A single strain of the feline virus has been reported [26]. The P150 (mice) or P145 (humans) c-*abl* protein exists in two predominant forms which differ at the amino terminus and are not separable in SDS-PAGE [10,27,28]. Altered forms of the human gene product, the P210 and P185 proteins, are encoded by the *bcr/abl* gene fusions associated with chronic myelogenous leukemia (CML) and some acute lymphocytic leukemias (ALL) [22,29–34]. Although *abl* genes have been described in both nematodes and *Drosophila*, *abl* protein has been identified only in *Drosophila* [35].

II-B. Response of cells to abl proteins

Although all *abl* proteins are tyrosine kinases, the ability of the three basic types to affect cellular growth is vastly different. For example, c-*abl* proteins, encoded by the normal cellular gene have no demonstrated effect on cell growth, even when expressed at high levels from various recombinant constructions [36,37]. The v-*abl* proteins induce transformation in a variety of cell types [4] while the *bcr/abl* proteins stimulate the growth of certain types of hematopoietic cells in vitro [38,39].

Much of the work on the transforming potential of *abl* has centered on studies of Ab-MLV. This virus transforms a narrow spectrum of fibroblastic cell lines including NIH 3T3 [4]. Other fibroblasts, such as BALB 3T3, and primary mouse embryo fibroblasts are refractory to transformation [5,40]. At least part of this differential susceptibility is mediated by a lethal effect of v-*abl* protein [40,41] that may reflect an inability of some cells to tolerate high levels of the v-*abl* kinase. Whether this phenomenon accounts for the failure of Ab-MLV to induce sarcomas in mice is not clear. However, the observation that the v-*abl* protein encoded by HZ-2 virus induces sarcomas in cats and pre-B lymphomas in mice [42] suggests that these differences are not inherent to the *abl* protein.

Infection of bone marrow in vitro with both the murine and feline forms of v-*abl* induces transformation of pre-B cells [7,42]. Expression of a kinase active v-*abl* protein is required for both initiation and maintenance of the transformed state in these cells (Ref. 43 and Engelman, A., and Rosenberg, N., unpublished data). Despite these observations, isolation of transformants using stromal cells reveals that changes in addition to v-*abl* expression are required for a fully transformed phenotype [44]. Similar events are likely to be required in vivo because Ab-MLV-induced tumors are clonal [45,46]. Thus, while v-*abl* expression is required for both initiation and maintenance of transformation, this signal alone is not sufficient for oncogenesis. A possible role for insertional mutation, mediated by the helper virus present in some Ab-MLV stocks has been suggested [47], but the gene(s) activated in this process has not yet been identified.

Most Ab-MLV infections induce pre-B cell lymphomas. However, thymomas, mastocytomas and plasmacytomas can be induced under some circumstances [4]. The ability of v-*abl* protein to substitute for IL-3 and at least sometimes IL-2 [48–51] may be important in some of these responses. The exact mechanism by which the v-*abl* protein allows cells to bypass lymphokine requirements is not known. While it is clear that the *abl* protein is not the IL-3 receptor, the latter molecule appears to be phosphorylated on tyrosine [52], suggesting that *abl* and IL-3 may share common intracellular signaling pathways. The ability of v-*abl* to substitute for erythropoietin and stimulate erythroid differentiation [53], although involving a different receptor system, may reflect a similar type of intracellular interaction.

Although the *bcr/abl* protein is associated with several human malignancies, expression of this protein does not usually directly transform cells in vitro [4,38,39,54]. Transgenic animals carrying a *bcr*/v-*abl* gene do develop tumors, but the latent period is quite long and the frequency of tumors is low [55]. Retroviral constructs expressing either the P210 or P185 form of *bcr/abl* stimulate the clonal outgrowth of pre-B cells in Whitlock-Witte cultures [38,39], but these cells acquire a fully malignant phenotype only after extended culture. In contrast, infection of bone marrow from mice treated with 5-fluorouracil, a drug that stimulates cycling of hematopoietic stem cells [56,57] reveals that *bcr/abl* can directly transform pre-B cells (Kelliher, M., McLaughlin, J., Witte, O.N. and Rosenberg, N., unpublished data). For reasons that are not understood, the P185 form of *bcr/abl* appears to transform cells more efficiently in both of these assays (Ref. 39 and Kelliher, M., McLaughlin, J., Witte, O.N. and Rosenberg, N., unpublished data). Despite an association with myeloid disease in humans, attempts to demonstrate an effect on cells of this lineage by *bcr/abl* protein either in vitro or in transgenic mice have not succeeded (Ref. 55, 58 and unpublished data as above). This result is particularly surprising because murine v-*abl* does stimulate the malignant outgrowth of myeloid cells when bone marrow from 5-fluorouracil-treated mice is used as a target cell population (Kelliher, M. and Rosenberg, N., unpublished data).

Despite the differences in response of cells to v-*abl* and *bcr/abl* proteins, some similarities exist. For example, *bcr/abl* protein can substitute for IL-3 in some cell systems [59,60]. The protein can also rescue the transformed phenotype of pre-B cells carrying temperature sensitive (*ts*) v-*abl* proteins (Engelman, A. and Rosenberg, N., unpublished data). Both of these results suggest that *bcr/abl* and v-*abl* affect similar signaling pathways once transformation is established and argue that *bcr/abl* differs from v-*abl* in its ability to initiate the transformation process.

III. Domains of the *abl* proteins

III-A. Overview

Although research efforts have identified a number of *abl* gene products and described their properties, a clear understanding of the way in which the different forms achieve their impact on cellular growth is still lacking. Analysis of cDNA clones and cloned v-*abl*-containing viruses has allowed comparison of the deduced protein sequence of the various Abelson proteins. Thus, differences between both the forms of the c-*abl* proteins and the c-*abl* and v-*abl* proteins are clearly delineated. However, the functional significance of each of these differences is only partly understood. Unraveling these mysteries remains one of the major challenges in this area of research.

III-B. The catalytic domain

The catalytic or SH_1 domain (*src* homologous domain 1), defined as amino acids 240 through 500 [61], is required for enzymatic function and is the portion of the *abl* proteins that is most conserved between the various forms. (Throughout, unless stated otherwise, amino-acid numbers are deduced using the sequence containing information from human exon 1A.) For example, the murine v- and c-*abl* proteins are identical within the catalytic domain [62–64]. Comparison of the murine and human c-*abl* proteins in this region reveals only four substitutions; one of these is a conservative change [28]. As expected for a functionally important portion of the molecule, the human proteins show 89% similarity and 77% identity with the *Drosophila* protein [28,35] and share about 60% of the residues with *C. elegans* protein [65].

The catalytic domain of the *abl* proteins shares many features with other protein kinases, including those with serine specificity [61]. Virtually all of these molecules have a lysine, corresponding to Lys-271 in the human *abl* protein, preceded by the consensus Gly-X-Gly-X-X-Gly. Although not formally shown for *abl*, by analogy to other kinases, the lysine is probably involved in proton transfer following interaction with ATP; the glycine-rich consensus, located 17 residues earlier in *abl* proteins, is probably involved in nucleotide binding [66,67]. Other landmarks of protein kinases found in the catalytic domain include additional residues that may be involved in ATP binding, such as Asp-363 and Asn-368 and the Asp-Phe-Gly stretch at positions 381 to 383. The Ala-Pro-Glu triplet at positions 407 to 409 is also a hallmark of these enzymes [68].

Although *abl* shares many features with all protein kinases, the relationship to those with tyrosine specificity is especially close. Among oncoproteins, *abl* is most similar to *fes* [61]. However, strong homologies to other cytoplasmic tyrosine kinases, especially those in the *src* family, are also evident. For example, two stretches near the residues implicated in ATP binding that may be important in determining tyrosine specificity are found at positions 363 through 368 and 402 through 409 [61]. Many of the tyrosine kinases also share a major phosphorylation site, corresponding to Tyr-393. Whether phosphorylation at this site is an important mediator of catalytic activity remains unclear. The corresponding site is phosphorylated on v-*abl* and *bcr/abl* but not c-*abl* proteins in vivo [23], suggesting that this difference may be important for protein function. However, experiments mutagenizing the homologous residue in *src* proteins have not uncovered a central role for this phosphorylation [67,70].

Consistent with the importance of the catalytic domain to v-*abl* function, linker insertion mutations in this region generally abolish kinase activity and transforming potential [21,71]. Because two v-*abl* proteins lacking the last 8 or 10 residues of the catalytic domain transform cells, the entire catalytic domain is not required for this function [42,72]. A linker insertion mutation just four amino acids further into the catalytic domain abolishes both transforming potential and kinase activity [71]. Together, these data define the maximum sequence requirement for transformation at the carboxyl-terminal end of the catalytic domain.

Some mutations within the catalytic domain create *ts* v-*abl* proteins. Insertion of a methionine after residue 327, a valine and a proline after residue 339 as well as substitution of a histidine at position 456 or a glycine at position 415 have this effect [72,73]. Although none of these mutations involves residues implicated in specific functions, they all affect residues that are conserved in most members of the *src* subfamily of protein tyrosine kinases [61]. Combining the two substitution mutations confers *ts* kinase activity to *bcr/abl* proteins (Rosenberg, N. and Witte, O.N., unpublished data).

III-C. The SH_2 domain

Between the *gag*-derived sequences and the stretch of amino acids that define the catalytic domain, both v-*abl* proteins contain a region that is highly conserved among all *abl* proteins [35,42,62–65] and shared with other tyrosine kinases [74]. This portion of the molecule, called SH_2, comprises 125 amino acids in the v-*abl* proteins and contains two subregions called B and C. In addition to being conserved in most tyrosine kinases, these subregions are also found in the v-*crk* oncoprotein, the GTPase-activating protein (GAP) and phospholipase CII (also called gamma) [74–78]. These latter proteins are not tyrosine kinases, but at least *crk* and phospholipase CII may be involved in signaling pathways involving these enzymes (reviewed in Ref. 78).

The function of the B and C subregions in any of the diverse molecules that contain them is not known. Current models suggest that these subregions may be involved in protein–protein interactions occurring during signaling or that they are important regulatory regions. Expression of v-*crk* induces transformation and an elevation of cellular phosphotyrosine [76], suggesting that the v-*crk* protein interfaces with growth pathways that are modulated by tyrosine phosphorylation. However, the role of the B and C sequences in v-*crk*-mediated transformation has not been addressed directly. In the case of GAP and phospholipase, neither region appears to be strictly required for activity [78,79]. Linker insertion mutations affecting the SH_2 domain of v-*abl* protein do not affect the ability of Ab-MLV to transform NIH 3T3 [21,71]. However, a mutation removing this region and the last 49 amino acids of *gag* yields a non-transforming virus [21] as do some mutations affecting the SH_2 domain in other oncogenic tyrosine kinases [74,80].

III-D. The SH_3 domain

The SH_3 domain is present in c-*abl* proteins and in the feline form of v-*abl*. This region borders the B subregion of the SH_2 domain and contains a third subregion called A. These sequences are also shared with v-*src*, v-*crk* and phospholipase CII [75,76]. However, while all c-*abl* proteins are very closely related in this area, conservation with other oncoproteins in this region is less striking than observed in the B and C subregions. Nonetheless, this region appears critical for normal c-*abl* function. Analysis of a series of c-*abl* deletion mutations expressed from retroviral LTRs has revealed that constructs lacking 54 amino acids, including those related to the A region, transform NIH 3T3 and induce lymphomas in mice [36,37]. The activation

of transforming potential is not directly correlated to protein kinase activity but does correlate with the ability of the c-*abl* protein to phosphorylate itself on tyrosine in vivo [36]. While overproduction of the 'activated' form is probably also important for transformation [36,37], a change in localization to exclusively cytoplasmic appears to be of central importance (Baltimore, D., personal communication). Because both the *bcr/abl* and feline v-*abl* proteins contain the residues absent in the transforming c-*abl* mutants [42,81,82], their function can apparently be repressed in the presence of either *bcr* or *gag* sequences.

III-E. The amino terminus

Unlike the other portions of *abl* protein, the various forms of the molecule differ drastically at the amino terminus. The c-*abl* genes in mammalian cells encode at least two protein forms with very different amino termini. Both forms of v-*abl* protein derive these sequences from retrovirus *gag* genes while the *bcr* gene contributes these sequences in the case of altered *abl* proteins associated with CML and ALL. These sequence differences probably play a key role in the function of the various *abl* proteins, affecting kinase activity or substrate interaction or both.

The sequences at the extreme amino terminus of c-*abl* protein define specific forms of the c-*abl* product [27,28]. Sequence analysis of human and murine c-*abl* cDNA clones predicts that one form of the molecule, called Type I (murine) or 1A (human) has 26 unique amino acids at the amino terminus. The Type I sequence is identical in the human and murine proteins and does not contain signals for membrane localization. The Type 1 protein may be the predominant cytoplasmic form of c-*abl* [23].

The second major form of c-*abl* protein, called Type IV (murine) or 1B (human) contains a 46-residue amino-terminal region. Two conservative substitutions distinguish the murine and human forms [27,28]. This form contains an N-terminal glycine, identical to the myristylated residue present in the *gag* derived MA (p15) proteins [83] and recent work has shown that this form of the protein is indeed myristylated [37]. The Type IV form is probably the predominant membrane associated form of the molecule. In addition, a significant amount of this form is localized to the nucleus (Baltimore, D., personal communication). Because nuclear localization is lost in all transforming *abl* proteins (Baltimore, D., personal communication), this property of the type IV molecule is probably central to its function.

In addition to the Type IV and Type I murine proteins, analysis of cDNA clones predicts that two other forms of the protein may exist. These molecules, called Type II and Type III, have amino-terminal regions of 20 and 21 residues, respectively [27]. Evidence for the presence of these forms in humans or other species has not been described. Direct study of the Type II and III proteins has been hampered by the low abundance of the mRNA encoding the Type III form and an inability to detect mRNA for the type II form [27,84]. Thus, the significance of these two products remains to be determined.

The functional importance of the amino terminal region of c-*abl* proteins is reinforced by the homology observed between the mammalian and the *Drosophila* protein in this region. The extreme amino terminus shares homology with the Type 1B human protein and retains an N-terminal glycine that could be myristylated [35]. While evidence for a myristylated form has not been presented, chimeric human Type 1B c-*abl*/*Drosophila* genes can rescue flies carrying *abl* mutations (Hoffman, F.M., personal communication), demonstrating functional similarity of these two regions. Thus far, no evidence for a form of the protein homologous to mammalian Type 1 has been found [35]. Because human Type 1A/*Drosophila* chimeric genes do not rescue mutant *abl* alleles (Hoffman, F.M., personal communication), this form of the protein, if present, is unlikely to be functionally important.

Both v-*abl* proteins derive their amino termini from retrovirus *gag* sequences [8,9,26,42]. The feline form probably contains 344 amino acids specified by this gene, while the first 236 residues of the murine v-*abl* protein are derived from *gag* information [42,62]. The entire MA (p15) and p12 (p10 in the feline virus) proteins and a portion of the CA (p30) protein are present in the v-*abl* proteins. In replication competent viruses, the *gag* sequences are normally processed to yield virion structural proteins [85]; processing does not occur in either of the viral *abl* proteins [8,86]. The MA protein, which is important for membrane localization of the normal retrovirus *gag* precursor polypeptide [83,85], probably provides a similar function in the *abl* protein. The function of the p12 protein in replication competent viruses is still obscure and the role of these sequences in the *abl* proteins is not completely understood. In at least some cases, a portion of this region appears to be important for protein stability in particular cell types [87,88]. No role for the CA protein sequences has been proposed. However, because several feline viruses containing different *onc* genes contain similar amounts of CA [42,89], these sequences may be important for the biology of the virus.

The *bcr/abl* proteins derive their amino terminal amino acids from the *bcr* gene. The P210 form contains 937 *bcr*-encoded residues [81,90]; the P185 form appears to share the first 455 amino acids with P210 [91,92]. These sequences are not related to either *gag* or normal c-*abl* sequences. Consistent with the absence of an N-terminal glycine that could serve as a target for

myristylation [81,90,92], the *bcr/abl* protein is predominantly cytoplasmic. The P185 and P210 forms of *bcr/abl* usually contain identical c-*abl* sequences, beginning with residue 27, the first residue specified by exon 2. The sequence differences in the amino-terminal region of the two forms of *bcr/abl* protein may account for the more efficient transforming activity of the P185 form in Whitlock-Witte cultures [39]. Alterations in kinase activity or differences in substrate interactions may be responsible for this phenomenon. The ability to purify large amounts of *bcr/abl* protein using baculovirus vectors [93] should help address these issues.

III-F. The carboxyl terminus

In contrast to many protein tyrosine kinases, the catalytic domain of the *abl* proteins is toward the amino-terminal end of the molecule. The function of the long, 630 residue carboxyl-terminal region is not completely understood. This region is not required for enzymatic activity, in either eukaryotic cells [24,71,87] or *E. coli* [17,71]. Several chimeric mice carrying one c-*abl* allele encoding a protein with a truncated carboxyl terminus have been bred. Preliminary studies reveal that these animals are normal, indicating that loss of the region is not a dominant mutation (Goff, S., personal comunication). However, except for the feline v-*abl* protein [42], all c-*abl* proteins [12,25,28,35,64] and the naturally occurring murine v-*abl* proteins [62,63] share this region, suggesting that it is of functional significance.

Conservation within the carboxyl-terminal portion of *abl* proteins is not as precise as that seen in other portions of the molecule. Analysis of one c-*abl* cDNA clone suggests that two mutations in the P160 genome, the deletion of a G at position 2915 and the insertion of a G at position 2982, result in the insertion of 23 unique amino acids at a position equivalent to residues 645 through 668 in human c-*abl* [63,64]. A point mutation at base 3477 results in the substitution of a glutamic acid for Lys-832 [63,64]. While the significance of these changes has not been assessed directly, data obtained with a variety of Ab-MLV strains and c-*abl* expression systems suggest that they are not central to protein function.

Comparison of the human and murine c-*abl* proteins in the carboxyl terminal region reveals a significant number of mismatches. Several short gaps are required to align the two sequences; about 75% of the amino acids are identical and 88% are conserved [28,64]. The region immediately following the catalytic domain (residues 501 to about 770) and a second region from about residue 990 to the end of the molecule are the most closely related. Serine residues that are phosphorylated by protein kinase C are contained within each of these regions [94]. However, whether these phosphorylations are important for transformation or kinase activity remains unclear. A stretch of five lysine residues between amino acids 605 and 611 are also conserved. This region appears to interact with the SH_3 domain of the Type IV c-*abl* protein and direct a portion of this form of *abl* protein to the nucleus, an interaction that is probably important for normal function (Baltimore, D., personal communication).

The *Drosophila* c-*abl* gene product diverges from the mammalian proteins throughout the carboxyl terminal region. Long stretches of homology do not exist and the *Drosophila*-encoded molecule is 234 residues longer [35]. Like the mammalian proteins, the carboxyl-terminal portion of the molecule is proline rich; about 18% of the residues in the mammalian proteins and 14% of those in the *Drosophila* protein are prolines. The possible function of these proline-rich stretches remains obscure. However, since the entire carboxyl terminus is dispensible for fibroblast and in some cases lymphoid transformation, a straightforward explanation is not likely.

A variety of mutations affecting the carboxyl-terminal region of v-*abl* proteins has been constructed or isolated using biological selection [24,87,95,96]. Although the properties of these viruses are somewhat confusing, it seems clear that the lack of an intact carboxyl terminal region does not affect transformation of NIH 3T3 cells [87,95]. However, a complete carboxyl-terminal region is associated with lethality in some fibroblast systems [40,41,95]. Many mutations that cause the loss of large parts of the carboxyl-terminal region affect the ability of the protein to transform lymphoid cells in vitro and in vivo [87,88,96, 97]. However, Ab-MLV strains encoding *abl* proteins that terminate before the second carboxyl-terminal proline-rich region retain their ability to induce Abelson disease, but are severely compromised in their ability to transform lymphoid cells in vitro [96,98].

IV. The structure and expression of c-abl genes

IV-A. Overview

Keys to understanding the role of c-*abl* in normal differentiation and to elucidating the mechanism by which its normal function is subverted in the viral form have come from molecular analyses of *abl* genes. Cloning of the major portion of the human and murine c-*abl* genes as well as the related *abl* genes from *Drosophila melanogaster, Calliphora erythrocephala,* and *Caenorhabditis elegans* has revealed basic relationships between these loci and allowed manipulation of the sequences in recombinant DNA based expression systems [12,28,35,65,99–101]. The isolation of *abl* genes in lower eukaryotes may pave the way for genetic analyses that directly address gene function. Finally, clues to the elements important in controlling c-*abl* expression have come from sequencing of cDNA clones [27,28,35,64].

IV-B. The human and murine c-abl genes

The human and murine c-*abl* genes are large units, spanning greater than 230 and 150 kilobases, respectively [99,100]. These genes appear to have twelve exons. There are multiple forms of the 5′ most exon which are collectively termed exon 1, but each c-*abl* mRNA contains sequences from only one of the forms [27,28]. Exons two through twelve are sometimes referred to as common exons because all c-*abl* mRNAs contain sequences transcribed from them. The precise organization of all of the common exons has not been mapped. However, hybridization experiments using cDNA probes and heteroduplex analyses have demonstrated a similar pattern for both the human and murine c-*abl* genes [12,28].

Considerable effort has focused on the 5′ region of the gene in an effort to understand the features controlling expression and exon 1 usage. The human gene has two exon 1 units, termed 1A and 1B. Exon 1A is 19 kb upstream of the first common exon (exon 2) and encodes the type 1A form of c-*abl* protein [28,100]. Exon 1B is at least 200 kb upstream of exon 1A and encodes the type 1B form of c-*abl* protein [28,100]. Definitive experiments identifying promoter and possible enhancer elements have not been reported. However, both forms of exon 1 have upstream G-C rich regions and no TATA or CAAT motifs have been found in the exon 1A promoter. The exon 1B promoter does contain a single CAAT motif 59 bases upstream of the sequence TTTAAAAGG [100]. This latter sequence resembles the TATA element of the rabbit β-globin gene [102]. However, these sequences are not conserved in the murine gene, making it likely that the promoters for both exons are contained within the G-C rich regions. These regions contain multiple GGGCGG repeats [28,100] which are potential binding sites for the transcription factor Sp1 [103].

The murine c-*abl* gene contains four distinct exon 1 units, called types I–IV after cDNA clones containing these sequences [27,99]. The murine type I exon shares homology with human exon 1A and the murine type IV exon is similar to human 1B. In addition to sequence homology, the position of these exons within the locus is conserved between the two species. Exon 1, type I is about 17 kb upstream of the first common exon, while the type IV unit is at least 90 kb upstream of this point [99]. The murine type II and type III exons have no known counterpart in the human genome [28,100]. The type III unit is located just downstream of the type IV exon, while the type II sequences are approx. 400 bp downstream of the type I exon [99].

As in the case of the human c-*abl* gene, promoter regions rich in G-C are located upstream of both the type I and IV forms of exon 1. For each form, transcription appears to initiate at multiple points and both of the promoter regions contain potential Sp1 binding sites and lack TATA motifs [99]. The type III and type II exons do not have separate promoter elements and may be expressed by alternative splicing from the promoters of the type IV and type I exons respectively. The type II region contains repetitive sequences and transcripts containing these sequences have not been detected in any cell type [27,84,99]. Thus, the original cDNA clone in which the type II exon was identified may have arisen via aberrant splicing and these sequences may not be functional.

IV-C. Expression of c-abl in murine and human cells

Analysis of cell lines and tissues has revealed that c-*abl* RNAs of 6.0 kb (human) and 5.3 (murine) contain sequences from exon 1A (type I) while those of 7.0 (human) and 6.0 (murine) contain sequences from exon 1B (type IV) [25,28,84]. The factors modulating alternative usage of the forms of exon 1 have not been elucidated. However, the two RNAs direct synthesis of distinct proteins that probably play different roles in cellular growth and metabolism. Thus, identifying the control mechanisms involved in exon 1 usage may provide clues to understanding c-*abl* expression. The mechanism by which the splicing reaction deals with the great distance between exon 1B and the first common exon also poses an intriguing question. Indeed, based on estimates of the rate at which RNA polymerase transcribes DNA sequences [104], at least 2 h would be required to traverse the entire c-*abl* locus [100].

While most tisues express c-*abl*, the RNA is especially prominent in testis and lymphoid tissues, particularly thymus [25,84,105]. The relative abundance of the two transcripts has been assessed for a number of murine tissues, revealing that differences in the two-fold range can be detected [84]. The RNA containing exon 1A sequences is highest in heart, muscle, spleen and thymus and lowest in liver and brain. The RNA containing exon 1B sequences is relatively constant from tissue to tissue. Type III RNA accounts for a small percentage of the total *abl* RNA and has been found in all tissues tested. The biological significance of the variations in abundance of the different forms of the RNA remains to be determined. As noted earlier, type II RNA has not been detected [27,84], suggesting that, if this form exists, it is present in very low abundance.

In addition to the two common c-*abl* RNAs, mouse testis expresses a unique, 4.0 kb species of c-*abl* RNA [64,105,106]. This RNA is first detected in populations enriched for early spermatids and is still prominent in late spermatids [106]. Both of these cells are post-meiotic suggesting that c-*abl* may play a specific role in the late differentiation stages of sperm. Analysis of a testis-derived cDNA clone reveals that this RNA contains exon 1 type I sequences and is identical to other c-*abl* RNAs

with respect to coding information [64]. Conclusive data concerning the use of exon 1 type IV sequences in a testis specific RNA have not been presented. The 4.0 kb RNA is polyadenylated at a site lacking obvious signals for polyadenylation upstream of the usual site and thus lacks 3′ sequences presumed to be untranslated in other c-*abl* RNAs [64]. The missing region contains a small open reading frame present in other c-*abl* RNAs that could direct synthesis of an 18 kDa peptide. However, no evidence for the existence of this peptide has been presented.

IV-D. arg – an abl-related gene

A portion of a second human gene called *arg* (*abl*-related gene) has been isolated from a genomic DNA library [107] using a v-*abl* probe from the region encoding the tyrosine kinase domain. The clone appears to contain two exons that share about 70% homology at the nucleic acid level with corresponding sequences in human c-*abl*. When the putative protein product is compared to the c-*abl* protein, 92% homology is found [28,107]. This gene is not linked to c-*abl* and has been localized to chromosome 1q24-25. Information on *arg* homologues in other species has not been presented. An *arg*-specific 12 kb RNA has been detected in various human cell lines suggesting that the product of the gene may be widely expressed. However, no information on the protein, presumably a protein tyrosine kinase, is available. Although *arg* and c-*abl* clearly are closely related in the regions of the gene that can be compared, a clear picture of their relationship awaits cloning of larger segments of the *arg* gene or analysis of *arg* cDNA clones.

IV-E. abl genes in other eukaryotes

Hybridization studies have identified *abl*-related genes in dipterans (flies) [101,108] and in the nematode, *C. elegans* [65]. Homologues of *abl* have not been detected in yeast. Among the *abl* genes found in lower eukaryotes, the *Drosophila* gene has been examined in the most detail. This gene contains ten exons and spans greater than 26 kb in the 73B region of the third chromosome [35,109]. Although the exact structure of the gene is distinct from the mammalian genes, there are several common features. For example, the first exon is separated from the other exons by considerable distance, about 14 kb, and the 3′ most exon is large. Unlike the mammalian genes, only one form of exon 1, a unit that shares homology with the mammalian exon 1B has been detected [35].

In *Drosophila*, *abl* RNA can be detected in unfertilized eggs and during embryogenesis and metamorphosis [110,111]. The RNAs range from 6.5 to 7.5 kb, with size variation arising from the use of both alternative splicing sites in the 3′ most intron and several polyadenylation sites [111]. Early in embryogenesis, the *abl* RNAs appear to be of maternal origin and are found uniformly in the embryo. The first zygotic expression begins about 8–12 h after fertilization, at which point the transcripts are detected in the area of the ventral nerve cord and the brain (Hoffman, F.M., personal communication). Localization studies using anti-*abl* antibodies have shown that the protein, like the RNA, is present in all cells in the blastoderm. The protein is detected coincident with zygotic expression 8 h after fertilization in axons within the CNS. Later, in the larval stages, *abl* protein is found only in those epithelial cells of the imaginal discs which give rise to the eyes and in both the cell bodies and axons of the retinal cells.

Analyses of *Drosophila* carrying mutant *abl* alleles have revealed that the gene is required for the adult fly to emerge from the pupal case [109]. This phenotype may suggest that c-*abl* is required for the co-ordinated neuronal development required to escape the pupal case. Study of flies with *abl* mutations has failed to reveal such a function. However, analysis of flies carrying mutant *abl* alleles and heterozygous for mutations in the unrelated *disabled* gene reveals that *abl* plays a key role in axonal development in embryogenesis [112]. A percentage of flies carrying some *abl* mutations emerge from the pupal case. These animals are weak, sterile and have eye defects stemming from disorganization or the lack of the retinal cells [109]. This phenotype is consistent with the expression of *abl* during formation of the eyes.

V. The structure of v-*abl* genes

V-A. Overview

Two viral forms of *abl* have been identified, one in the murine virus Ab-MLV [1] and the second in HZ-2 virus, a feline virus [26]. The murine virus contains sequences from murine c-*abl*, while feline c-*abl* sequences were transduced to generate HZ-2 [12,42]. The precise recombination points between viral and c-*abl* sequences and the amount of c-*abl* sequence present differ in the two viruses. Neither virus contains the entire c-*abl* coding sequence. However, both contain the portion of c-*abl* that encodes the tyrosine kinase domain of the *abl* protein. In each case, the 5′ recombination involved sequences in the *gag* gene of the retroviral parent and sequences in a c-*abl* exon. Thus, the amino terminal portion of the *abl* protein encoded by the viruses contains sequences derived from a gene that normally encodes virion structural proteins. Finally, control of v-*abl* expression is mediated by promoter, enhancer and polyadenylation signals present in the viral LTRs. Each of these features probably plays a key

role in the ability of the v-*abl* genes to induce cellular transformation.

V-B. Ab-MLV

Ab-MLV arose in a single BALB/c mouse injected with Moloney murine virus (Mo-MLV) [1]. Sequence analysis of cloned proviral forms has shown that the recombination which generated the virus occurred after base 1329 of the viral genome, within the sequences encoding the CA (p30) protein [62]. The c-*abl* recombination point is within the third c-*abl* exon [12]. As a consequence of the recombination, 236 *gag* codons have replaced 26 common exon codons and all of the codons derived from exon 1 at the 5′ end of the gene. A small, four base homology shared by Mo-MLV and c-*abl* at the recombination point may have been important in mediating the recombination event [12]. The 3′ recombination point involves sequences at the extreme end of the Mo-MLV *env* gene and sequences in the 3′ untranslated region of the last c-*abl* exon [12,62]. Thus, the virus lacks a stretch of 765 bases from the 3′ untranslated region of c-*abl*. No obvious homology exists between these regions involved in the 3′ recombination.

V-C. HZ-2 FeSV

The HZ-2 isolate of feline sarcoma virus was isolated from a spontaneous multicentric fibrosarcoma arising in a Siamese cat [26]. The 5′ recombination point in HZ-2 involves sequences in the portion of the feline leukemia virus (FeLV) *gag* encoding the CA (p30) protein and sequences in exon 2 of feline c-*abl*. As a consequence, the feline form of v-*abl* contains 63 c-*abl* codons not present in Ab-MLV. Striking homology is not shared at the recombination point. However, the recombination point in *gag* is within 24 bases of the recombination point involved in generating four other unrelated feline viruses that contain *onc* genes distinct from c-*abl* [89]. Thus, recombination within this region of the *gag* gene may be functionally important.

While the 5′ recombination point that generated HZ-2 occurs upstream of the Ab-MLV recombination, the 3′ recombination point occurs after only 1317 bases of c-*abl* sequence [42]. This recombination involves sequences in the FeLV *pol* gene and c-*abl* sequences near the end of those encoding the catalytic domain of the *abl* protein. Thus, feline v-*abl* shares only 316 codons with the murine form. The 3′ recombination point generates an in-frame fusion with *pol* coding sequences. While the complete sequence of HZ-2 downstream of the recombination point is not available, as many as 66 *pol* codons may contribute to the v-*abl* protein encoded by this virus [42].

VI. Altered *abl* structure and expression in human leukemias

VI-A. The Philadelphia chromosome and the c-abl gene

The Philadelphia translocation t(9;22)(q34,q11) is found in over 90% of all cases of CML [113–116] and in a much smaller proportion of patients with ALL [117–119]. This chromosomal marker is generated by a translocation in which the terminal portion of the long arm of chromosome 9(q34) is joined to the truncated long arm of chromosome 22. A reciprocal product called the 9q + chromosome in which chromosome 22(q11) is juxtaposed to the remaining portion of chromosome 9 is also generated. Mapping of the human c-*abl* gene to chromosome 9(q34) [120] and to the Philadelphia chromosome [121,122] suggested that it might play a role in this disease. An altered c-*abl* gene product was found in CML cells arising from the fusion of c-*abl* sequences with those of a heretofore undescribed cellular gene called *bcr* [22,29,30,123–126]. The subsequent demonstration of a similar product associated with the Philadelphia chromosome in ALL cells [31–33] solidified the role of this gene in these diseases. However, despite a clear understanding of the basic features of altered c-*abl* expression, the exact points in the disease process that are affected by the gene and the mechanism by which expression alters cell growth remain elusive In addition, while differences in the exact structures of the translocations in CML and in many cases of ALL are well delineated, the way in which these differences impact on the distinct course of the two diseases is not understood.

VI-B. Philadelphia chromosome structure in CML and ALL

The Philadelphia translocation juxtaposes c-*abl* sequences from chromosome 9(q34) and *bcr* sequences from chromosome 22(q11) [121,122,127]. Analyses of genomic clones from the DNA of CML cells has revealed that breakpoints in the *bcr* locus are clustered in a 6 kb region called the breakpoint cluster region or bcr [125,128–131]. This region consists of five small exons designated exons one to five respectively from 5′ to 3′ [125]. The breakpoints that have been characterized in detail occur in intron sequences between either the second and third or third and fourth exons in the region [125].

In contrast to the clustering of *bcr* breakpoints, the breakpoint in the c-*abl* gene varies widely but usually occurs within the large intron separating exons 1B and 1A [100,130,132]. More rarely, the breakpoint appears to map further 5′, in the vicinity of exons 2 and 3 [120,127,129]. Although not examined in detail, the remainder of the c-*abl* gene does not appear to be

altered as a consequence of the translocation in most cases [127,132,133]. Amplification and internal rearrangement of c-*abl*, noted in the K562 cell line [133], are not features generally associated with the translocation [130,134].

The Philadelphia chromosome associated with ALL is cytogenetically indistinguishable from that found in CML [117–119] and as in CML, is generated by a recombination joining c-*abl* and *bcr* sequences [33,135–137]. However, in about 50% of adult Philadelphia chromosome-positive cases of ALL and in over 90% of juvenile cases, this rearrangement involves sequences toward the 3′ end of the first intron in the *bcr* gene [137–140]. In the remaining instances, the recombinations do not differ significantly from those occurring in CML in that they involve the breakpoint cluster region [126,136,141]. A possible difference between these latter recombinations and those associated with CML could be the presence of large deletions of *bcr* sequence extending beyond the breakpoint cluster region [136]. As is the case with the rearrangements associated with CML, the breakpoint in the c-*abl* sequence usually occurs in the large intron between exons 1B and 1A [137]. Some recombinations have occurred further 3′ in the intron between exons 1A and 2 [33,135].

Evidence concerning the factors influencing the formation of the Philadelphia chromosome is scant. Regions of homology between the *bcr* and c-*abl* genes, even in the areas involved in the recombination have not been noted [125]. However, *alu* repetitive sequences have been found in the vicinity of the breakpoints on both chromosomes in both the CML and ALL translocations [125,131,137,139], suggesting that such sequences may play a role in the recombination. A high frequency of the ALL recombinations examined in one study [139] are associated with a restriction fragment length polymorphism (RFLP). This RFLP, generated by the deletion of 1 kb at the 5′ end of the *alu* repetitive elements in the first *bcr* intron, maps within 10 kb of the breakpoints. Both normal and tumor DNAs carry the RFLP which occurs in approximately one third of all *bcr* alleles. Whether the deletion associated with the RFLP is important in directing the rearrangement requires further study.

VI-C. Expression of bcr/abl

A single 8.2 kb *bcr/abl* RNA species is present in fresh bone marrow cells and in cell lines derived from Philadelphia chromosome-positive CML patients [123,124,128,133]. In all cases examined, the RNA initiates at the 5′ end of the *bcr* sequences and contains most, if not all of the *bcr* exonic sequences up to the breakpoint. In most cases, the *bcr* sequences are joined to c-*abl* sequences at the normal exon 2 boundary and the transcript is identical to the normal c-*abl* mRNA from that point [28,81,82]. Because many of the recombinations occur 5′ of c-*abl* exon 1A [100,130,132], the absence of these sequences in the *bcr/abl* mRNA indicates that these sequences are removed by splicing. The mechanism by which this is accomplished may be similar to the one which removes exon 1A sequences from some normal c-*abl* mRNAs.

In the cases of ALL where the breakpoints are similar to those observed in CML, the same pattern of RNA expression is observed [31–33,136]. Cells from patients with rearrangements in the first *bcr* intron usually contain a 7 kb RNA that is the product of the *bcr/abl* fusion gene [33,91,92,135,138]. Although the translation of the mRNA may begin 120 bases earlier than in CML [92], most evidence suggests that, up to the breakpoint, the *bcr* sequences present in the fusion transcripts are virtually identical in CML and ALL [81,82,91,92]. As in the case of CML, the *bcr* sequences are fused to c-*abl* exon 2 sequences and appear to be colinear with normal c-*abl* mRNA 3′ of that point [91,92,135].

The 5′ untranslated region of *bcr/abl* appears to play a key role in translational control. This region is about 80% G-C and contains two stretches of G-C repeats of approx. 15 and 30 bp that are predicted to form stem-loop structures [81,90]. This region also contains two short open reading frames, one of which is preceded by the Kozak consensus sequence and the other of which is associated with a sequence related to the Kozak consensus. However, no evidence for translation of these sequences has been found [143]. Because truncations removing the G-C repeats increase *bcr/abl* translation in rabbit reticulocyte systems, while a mutation affecting the potential start codon of one of the small open reading frames does not (Ref. 143 and Witte, O.N., personal communication), it is likely that secondary structure in the 5′ untranslated region is critical in controlling translation of the *bcr/abl* RNA.

VI-D. Structure and expression of the normal bcr gene

Interest in the structure and function of the *bcr* gene stems from its participation in the translocation characteristic of CML and some ALLs. The normal *bcr* gene spans 130 kb of DNA and while the exact structure has not been mapped, the coding sequence is divided into about 20 exons [125,126]. Similar to the arrangement of c-*abl* exons 1B and 2, the first and second *bcr* exons are separated by a large intron [126,139]. Whether particular structural features facilitate processing of the long transcripts generated by such distances remains an open question. These features, if they exist, may also be critical for processing of the long transcripts specified by many of the *bcr/abl* genetic units.

The *bcr* gene is expressed in the form of two RNAs of 4.5 and 6.5 kb in size in a variety of cells, including fibroblasts, lymphoid and myeloid cells and HeLa cells

[90,125,144]. The relationship of the two RNA species to each other is unclear. Analyses with a variety of probes has failed to pinpoint the difference [90]. Because only a single species of *bcr/abl* RNA is found [124,128,133,142], the difference between the two normal *bcr* RNAs is likely to lie at the 3′ end.

The *bcr* cDNA sequence contains a single long open reading frame of 3813 bp that could encode a 1271 amino acid protein [90,125]. This molecule is relatively hydrophilic, with an excess of basic residues; no hydrophobic domains that characterize transmembrane regions are present. Homologies to known proteins have not been uncovered. Although *bcr*-specific antisera precipitate two protein forms of M_r 160000 and 180000 to 190000 [144,145], expression of *bcr* in bacteria or in eukaryotic cells using retroviral vectors leads to production of only the 160000 form [143]. While the relationship of these molecules to each other and to the two RNA species is unknown, it is possible that the *bcr* sequences used in the expression systems lack the information necessary to encode the larger protein form.

The *bcr* protein is phosphorylated in vivo but whether only serines or both serines and threonines are modified has not been settled [143–145]. In contrast to *abl* proteins, phosphorylation of tyrosine residues in the *bcr* protein does not occur. A serine kinase activity is associated with the *bcr* molecule [143,145]. However, whether the activity is intrinsic to the *bcr* protein or is the property of a co-precipitant requires further study. Because the *bcr* protein shares no homology with any known protein kinases [91,125], the activity in the immune complexes is probably not a property of the *bcr* molecule.

Three additional *bcr* related loci, called *bcr*2, *bcr*3 and *bcr*4 have been described [146]. These sequences are closely linked to each other and to the original *bcr* gene and map to the q11.2 region of chromosome 22. The *bcr*-related regions share seven 3′ exons and the corresponding introns with each other and with the originally described *bcr* gene [146,147]. Beyond this point the three *bcr* related sequences share a short region of homology that is not found in the original *bcr* gene. After this point, each diverges into unique sequence. The combination of homologous and nonhomologous regions suggests that the *bcr*-related genes may have arisen by insertion of the 3′ part of the *bcr* gene into a common locus that then underwent variable duplication. Information on expression of these loci has not been presented.

VII. The role of *bcr/abl* in CML and ALL

VII-A. The biology of CML

A basic review of the pathogenesis of CML is needed to discuss the possible mechanisms by which altered *bcr/abl* expression plays a role in the disease. The primary event in CML is believed to be the clonal outgrowth of a pluripotent hematopoietic stem cell bearing the Philadelphia chromosome [148]. Initially the disease is characterized by a chronic phase during which production of committed myeloid progenitor cells and occasionally platelet precursors is elevated. Although these cells exhibit subtle abnormalities in growth kinetics and in their requirement for growth factors [149–151], they undergo terminal differentiation and give rise to an increased pool of functional, mature cells. The chronic phase lasts between several months and several years, after which the disease enters the blast-crisis stage in which rapid proliferation of immature blast cells ensues (reviewed in Ref. 152). Although the blast cells most frequently belong to the myeloid lineage, lymphoblasts are found about thirty per cent of the time. More rarely, the blast cells belong to other hematopoietic lineages [152]. The involvement of multiple types of hematopoietic cells is consistent with the dominance of a single clone of hematopoietic stem cells.

Because the Philadelphia chromosome is present at the earliest clinically identifiable stages of the disease, it may be argued that *bcr/abl* expression is important for the initiation of the disease or for the early stages of progression or both. Expression of *bcr/abl* is definitely not restricted to the blast crisis stage [153,154] and is probably initiated as soon as the translocation occurs. If this supposition is correct, *bcr/abl* expression is probably the initiating event in CML and may be instrumental in inducing the clonal dominance of the stem cell carrying the translocation. Consistent with this interpretation, in vitro infection of normal murine bone marrow with retroviral constructs expressing *bcr/abl* stimulates the clonal outgrowth of lymphoid cells [38,58].

The Philadelphia chromosome is present in all descendants of the dominant stem cell clone. Thus, a perplexing question stems from the fact that proliferation of cells of the myeloid lineage is most often associated with the disease process [153]. It is possible that the *bcr/abl* protein is not expressed in all lineages or that differences in substrates or turnover rates vary in different cell types. Detrimental effects of the *abl* protein such as those seen in some fibroblast systems [40,41] or stimulation of normal differentiation as observed in fetal murine erythroid cells [53,155], may be important in determining the type of cells that proliferate.

The issue of cell specificity is complicated by observations made in murine model systems. Here, *bcr/abl* expression affects a cell not commonly involved in the chronic phase of CML, the lymphoid cell [38,58]. This outcome is not altered under conditions that select for either myeloid proliferation [58] or expression of the gene in stem cells (Kelliher, M., McLaughlin, J., Witte, O.N. and Rosenberg, N., unpublished data). This latter

result is particularly striking because v-*abl* expression does induce myeloid cell transformation under these conditions (Kelliher, M. and Rosenberg, N., unpublished data). Differences between murine and human cells may predispose to outgrowth of a cell type not commonly associated with the disease. Alternatively, the cell type involved in CML may be influenced by the hematopoietic microenvironment of the patient, a situation that may not be duplicated in the tissue culture model systems.

Understanding the changes involved in progression from the chronic to the blast-crisis phase of the disease is a central problem in CML. Because blast-crisis stage in an individual patient involves only cells of a single hematopoietic lineage, the change involved probably does not occur at the stem cell level. Despite the finding that certain cell lines derived during blast crisis have elevated levels of *bcr/abl* expression [133], such changes have not been observed consistently [156,157]. Indeed, loss of the Philadelphia chromosome and *bcr/abl* expression in the late stages of the disease has been observed [158]. Chromosomal abnormalities such as trisomy 8 and 19, formation of a second Philadelphia chromosome and isochromosome 17 [159] are associated with the blast-crisis phase and these changes may activate other oncogenes or affect tumor suppressor genes. Activated *ras* genes have been detected in some CML cases [160], but they are not a general feature and are unlikely central to the disease process. Although activation of the *myc* oncogene is not associated with blast crisis [161,162], co-expression of this gene and *bcr/abl* induces transformation of rat cells in vitro [163]. Other, as yet unidentified, oncogenes may function in a similar fashion to induce progression of the disease to the blast-crisis stage.

Similar to the situation in CML, secondary changes are required for full malignant transformation of murine bone marrow cells expressing *bcr/abl* [38]. The incidence and course of disease in *bcr*/v-*abl* transgenics [55] suggests that similar events are also needed in vivo. Consistent with these observations, *bcr/abl* expression fails to induce transformation of normal murine bone marrow cells in one step transformation assays (Ref. 38; Kelliher, M., McLaughlin, J., Witte, O.N. and Rosenberg, N., unpublished data). The nature of the additional signals important for malignant progression in any of these systems has remained elusive. Identifying the secondary changes associated with progression may be complicated by the possibility that more than one type of secondary change complements *bcr/abl*.

VII-B. The biology of ALL

ALL is the most common childhood leukemia and occurs more rarely in adults; 10–20% of these cases are characterized by the presence of the Philadelphia chromosome [117–119]. Although the presence of this marker is generally associated with a poorer prognosis (reviewed in Ref. 164), definitive information on the role of *bcr/abl* in pathogenesis is not yet available. The disease is a distinct clinical entity from CML and is characterized by the aggressive, malignant proliferation of a committed progenitor cell of the B or more rarely the T lymphocyte lineage (reviewed in Ref. 165). In contrast to CML, the blood picture is usually monomorphic and the malignant clone shows no potential for differentiation [166]. The nature of the malignant clone remains unsettled [166–169]. The observation that some ALLs appear to evolve into chronic-phase CML following treatment supports that notion that this clone is a stem cell [167]. However, in contrast to CML, where the translocation persists in all lineages even during remission, ALL patients induced to remission lose all blood cells carrying the Philadelphia chromosome, suggesting that the malignant clone in this disease is a committed progenitor cell [166–169].

The molecular characterization of *bcr/abl* gene fusions in ALL may provide clues to the long standing controversy surrounding the origin of the disease. In some ALL cases, the pattern of *bcr/abl* expression appears identical to that observed in CML [126,136,141]. The majority of such cases occur in adults [140,164], raising the possibility that some of them may be blast-crisis-phase CML that escaped earlier diagnosis. The instances cited earlier in which Philadelphia chromosome positive cells remain during remission [167] may also fall into this category. Most juvenile cases of ALL express a distinct *bcr/abl* protein from that found in CML [139,140,164] and these cases may represent de novo ALL.

The P185 *bcr/abl* protein most commonly associated with ALL lacks *bcr*-derived sequences that are present in the P210 protein commonly associated with CML. This difference arises from the distinct recombinations that usually characterize the two diseases and may indicate that particular breakpoints are preferentially generated in different types of cells. However, it seems more likely that the two different proteins affect the growth and differentiation of different types of cells. The spectrum of transformed cells found in ALL more closely resembles that observed with v-*abl* and *bcr/abl* [4]. However, both the P210 and P185 forms of *bcr/abl* appear to interact with the same cell type, an immature B cell precursor, in murine model systems [38,39]. In addition, the P185 form of *bcr/abl* has been observed in acute myeloid leukemia [170], a disease of myeloid cells occasionally associated with the Philadelphia chromosome [170,171]. Consistent with the aggressive nature of ALL, the P185 form of *bcr/abl* has more potent transforming activity than P210 in vitro in one model system [39]. The differences in *bcr* determinants between the two proteins probably account for the dif-

ference in transforming potencies, but a direct examination of this question has not been reported.

VII. Future directions

Progress in understanding the structure and expression of the various forms of *abl* genes has been rapid. The ability to purify large amounts of *abl* protein using baculovirus vectors or other expression systems should open the door to experiments probing the biochemical properties of these molecules and may also facilitate the identification of cellular molecules that interact with *abl* protein. The availability of large amounts of pure *abl* protein is also a first step towards crystallization and biophysical analysis.

Solving the mysteries associated with the way in which the altered forms of *abl* protein impact on cellular growth and differentiation remain a major challenge. The once firmly entrenched view that v-*abl* is a transforming gene acting only on early B lineage cells has been replaced by a broader picture in which the different forms of *abl* have diverse effects on various hematopoietic cells. Although protein tyrosine kinase activity is central to all of these responses, features of *abl* proteins that are unique to these molecules must also play a key role. The availability of both molecular approaches to mutagenesis and in vitro and transgenic systems in which to measure the effects of *abl* expression may speed the dissection of functional regions of the proteins. Because *abl* is one of a large family of genes encoding protein tyrosine kinases, advances in this area may also be expected to impact on our understanding of cellular metabolism in a broader way.

The coming years should bring an increased understanding of the role of altered *abl* expression in CML and ALL. The absence of an adequate tissue culture or animal model system to study the perplexing CML system remains a stumbling block. However, introduction of altered *abl* genes into murine stem cells followed by reconstitution of irradiated mice or the use of special bone marrow stromal cultures to grow infected hematopoietic cells hold promise. Advances in this area should not only shed light on the mechanism by which altered *abl* expression affects cellular growth but also may lead to a clear picture of the pathogenesis of these two important human diseases. Improved treatment regimens may also follow.

While emphasis is often placed on the abnormal situations, understanding the function of the normal c-*abl* gene is of equal importance. The ability to manipulate this gene in lower eukaryotes may help to unravel this question. Experiments to inactivate or compromise expression of normal c-*abl* in mice using embryonal stem cell technology may also prove enlightening.

Certainly, if the progress of the last decade can be matched in the next, our understanding of both normal c-*abl* and all of the altered forms of *abl* will be advanced significantly. However, despite the advances that have been made, the problems that remain are complex and their solution will require both creative and labor intensive efforts on the part of both physicians and scientists.

Acknowledgements

We are grateful to the members of the laboratory and to Dr. John Coffin for discussions and for critical reading of the manuscript. N.R. is supported by grants (CA 24220 and CA 33771) from the National Institutes of Health.

References

1 Abelson, H.T. and Rabstein, L.S. (1970) Cancer Res. 30, 2213–2222.
2 Bishop, J.M. and Varmus, H. (1985) in RNA Tumor Viruses, (Weiss, R., Teich, N., Varmus, H. and Coffin, J., eds.), pp. 249–356, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
3 Gross, L. (1970) Oncogenic Viruses. Pergamon Press, New York.
4 Rosenberg, N. and Witte, O.N. (1988) Adv. Virus Res. 35, 39–81.
5 Scher, C.D. and Siegler, R.S. (1975) Nature 253, 729–731.
6 Rosenberg, N., Baltimore, D. and Scher, C.D. (1975) Proc. Natl. Acad. Sci. USA 75, 1932–1936.
7 Rosenberg, N. and Baltimore, D. (1976) J. Exp. Med. 143, 1453–1463.
8 Witte, O.N., Rosenberg, N., Paskind, M., Shields, A. and Baltimore, D. (1978) Proc. Natl. Acad. Sci. USA 75, 2488–2492.
9 Reynolds, F.H., Sacks, T.S., Deobagkar, D.N. and Stephenson, J.R. (1978) Proc. Natl. Acad. Sci. USA 75, 3974–3978.
10 Witte, O.N., Rosenberg, N. and Baltimore, D. (1979) Nature 281, 396–398.
11 Goff, S.P., Witte, O.N., Gilboa, E. and Baltimore, D. (1980) Cell 22, 777–785.
12 Wang, J.Y.J., Ledley, F., Goff, S., Lee, R., Groner, Y. and Baltimore, D. (1984) Cell 36, 349–356.
13 Whitlock, C.A. and Witte, O.N. (1985) Adv. Immunol. 37, 73–98.
14 Goff, S. (1985) Proc. Exp. Biol. Med. 179, 403–412.
15 Witte, O.N., Dagupta, A. and Baltimore, D. (1980) Nature 283, 826–831.
16 Van de Ven, W.J.M., Reynolds, F.R. and Stephenson, J.R. (1980) Virology 101, 185–197.
17 Wang, J.Y.J., Queen, C. and Baltimore, D. (1982) J. Biol. Chem. 257, 13181–13184.
18 Hunter, T. and Sefton, B. (1980) Proc. Natl. Acad. Sci. USA 77, 1311–1315.
19 Witte, O.N., Goff, S., Rosenberg, N. and Baltimore, D. (1980) Proc. Natl. Acad. Sci. USA 77, 4993–4997.
20 Reynolds, F.H., Van de Ven, W.J.M. and Stephenson, J.R. (1980) J. Virol. 36, 374–386.
21 Prywes, R., Foulkes, J.G. and Baltimore, D. (1985) J. Virol. 53, 114–122.
22 Konopka, J.B., Watanabe, S.M. and Witte, O.N. (1984) Cell 37, 1035–1042.
23 Konopka, J.B. and Witte, O.N. (1985) Mol. Cell. Biol. 5, 3116–3123.

24 Rosenberg, N. and Witte, O.N. (1980) J. Virol. 33, 340–348.
25 Wang, J.Y.J. and Baltimore, D. (1983) Mol. Cell. Biol. 3, 773–779.
26 Besmer, P., Hardy, W.D., Zuckerman, E.E., Bergold, P., Lederman, L. and Snyder, H.W. (1983) Nature 303, 825–828.
27 Ben-Neriah, Y., Bernards, A., Paskind, M., Daley, G.O. and Baltimore, D. (1986) Cell 44, 577–586.
28 Shtivelman, E., Lifshitz, B., Gale, R., Roe, B.A. and Canaani, E. (1986) Cell 47, 277–284.
29 Konopka, J.B., Davis, J.L., Watanabe, S.M., Singer, J.W., Collins, S.J. and Witte, O.N. (1985) Proc. Natl. Acad. Sci. USA 82, 1810–1814.
30 Kloetzer, W., Kurzrock, R., Smith, L., Talpaz, M., Spiller, M., Gutterman, J. and Arlinghaus, R. (1985) Virology 140, 230–238.
31 Clark, S.S., McLaughlin, J., Crist, W., Champlin, R. and Witte, O.N. (1987) Science 235, 85–88.
32 Kurzrock, R., Shtalrid, M., Romero, P., Kloetzer, W.S., Talpas, M., Trujillo, J.M., Blick, M., Beran, M. and Gutterman, J.U. (1987) Nature 325, 631–635.
33 Chan, L., Karhi, K.K., Rayter, S.I., Heisterkamp, N., Eridani, S., Powles, R., Lawler, S.D., Groffen, J., Foulkes, J.G., Greaves, M.F. and Wiedemann, L.M. (1987) Nature 325, 635–637.
34 Walker, L.C., Ganesan, T.S., Dhut, S., Gibbons, B., Lister, T.A., Rothbard, J. and Young, B.D. (1987) Nature 329, 851–853.
35 Henkenmeyer, M.J., Bennett, H., Gertler, F.B. and Hoffman, F.M. (1988) Mol. Cell. Biol. 8, 843–853.
36 Franz, W.M., Berger, P. and Wang, J.Y.J. (1989) EMBO J. 8, 137–147.
37 Jackson, P. and Baltimore, D. (1989) EMBO J. 8, 449–456.
38 McLaughlin, J., Chianese, E. and Witte, O.N. (1987) Proc. Natl. Acad. Sci. USA 84, 6558–6562.
39 McLaughlin, J., Chianese, E. and Witte, O.N. (1989) Mol. Cell. Biol. 9, 1866–1874.
40 Ziegler, S.F., Whitlock, C.A., Goff, S.P., Gifford, A. and Witte, O.N. (1981) Cell 27, 477–486.
41 Goff, S.P., Tabin, C.J., Wang, J.Y.J., Weinberg, R. and Baltimore, D. (1982) J. Virol. 41, 271–285.
42 Bergold, P.J., Blumenthal, J.A., D'Andrea, E., Snyder, H.W., Lederman, L., Silverston, A., Nguyen, H. and Besmer, P. (1987) J. Virol. 61, 1193–1202.
43 Takemori, T., Miyazoe, I., Shirasawa, T., Taniguchi, M. and Graf, T. (1987) EMBO J. 6, 951–956.
44 Whitlock, C.A., Ziegler, S.F. and Witte, O.N. (1983) Mol. Cell. Biol. 3, 596–604.
45 Green, P.L., Kaehler, D.A. and Risser, R. (1987) Proc. Natl. Acad. Sci. USA 84, 5932–5936.
46 Green, P.L., Kaehler, D.A., Bennett, L.M. and Risser, R. (1989) J. Virol. 63, 1989–1994.
47 Poirier, Y., Kozak, C. and Jolicoeur, P. (1988) J. Virol. 62, 3985–3992.
48 Cook, W.D., Metcalf, D., Nicola, N.A., Burgess, A.W. and Walker, F. (1985) Cell 41, 677–683.
49 Pierce, J.H., DiFiore, P.P., Aaronson, S.A., Potter, M., Humphrey, J., Scott, A. and Ihle, J.N. (1985) Cell 41, 685–693.
50 Oliff, A., Agranovsky, O., McKinney, M.D., Murty, V.V.V.S. and Bauchwitz, R. (1985) Proc. Natl. Acad. Sci. USA 82, 3306–3310.
51 Cook, W.D., Fazekas de St. Groth, B., Miller, J.F.A.P., MacDonald, H.R. and Gabathuler, R. (1987) Mol. Cell. Biol. 7, 2631–2635.
52 Isfort, R.J., Stevens, D., May, W.S. and Ihle, J.N. (1988) Proc. Natl. Acad. Sci. USA 85, 7982–7986.
53 Waneck, G.L., Keyes, L. and Rosenberg, N. (1986) Cell 44, 337–344.
54 Daley, G.Q., McLaughlin, J., Witte, O.N. and Baltimore, D. (1987) Science 237, 532–535.
55 Hariharan, I.K., Harris, A.W., Crawford, M., Abud, H., Webb, E., Cory, S. and Adams, J.M. (1989) Mol. Cell. Biol. 9, in press.
56 Hodgson, G.S. and Bradley, T.R. (1979) Nature 281, 381–382.
57 Van Zant, G. (1984) J. Exp. Med. 159, 679–690.
58 Young, J.C. and Witte, O.N. (1988) Mol. Cell. Biol. 8, 4079–4087.
59 Daley, G.Q. and Baltimore, D. (1988) Proc. Natl. Acad. Sci. USA 85, 9312–9316.
60 Hariharan, I.K., Adams, J.M. and Cory, S. (1988) Oncogene Res. 3, 387–399.
61 Hanks, S.K., Quinn, A.M. and Hunter, T. (1988) Science 241, 42–51.
62 Reddy, E.P., Smith, M.J. and Srinivasan, A. (1983) Proc. Natl. Acad. Sci. USA 80, 3625–3627.
63 Lee, R., Paskind, M., Wang, J.Y.J. and Baltimore, D. (1985) in RNA Tumor Viruses (Weiss, R., Teich, N., Varmus, H. and Coffin, J., eds.), pp. 851–868, Cold Spring Harbor Laboratory, Cold Spring Harbor NY.
64 Oppi, C., Shore, S. and Reddy, E. (1987) Proc. Natl. Acad. Sci. USA 84, 8200–8204.
65 Goddard, J.M., Weiland, J.J. and Capecchi, M.R. (1986) Proc. Natl. Acad. Sci. USA 83, 2172–2176.
66 Sternberg, M.J.E. and Taylor, W.R. (1984) FEBS Lett. 175, 387–391.
67 Kamps, M.P. and Sefton, B.M. (1986) Mol. Cell. Biol. 6, 751–757.
68 Hunter, T. and Cooper, J.A. (1986) in The Enzymes (Boyer, P.D. and Krebs, E.G., eds.), pp. 191–246, Academic Press, Orlando, FL.
69 Snyder, M.A., Bishop, J.M., Colby, W.W. and Levinson, A.D. (1983) Cell 32, 891–901.
70 Cross, F.R. and Hanafusa, H. (1983) Cell 34, 597–607.
71 Rees-Jones, R.W. and Goff, S.P. (1988) J. Virol. 62, 978–986.
72 Engelman, A. and Rosenberg, N. (1987) Proc. Natl. Acad. Sci. USA 84, 8021–8025.
73 Kipreos, E.T., Lee, G.J. and Wang, J.Y.J. (1987) Proc. Natl. Acad. Sci. USA 84, 1345–1349.
74 Sadowski, I., Stone, J.C. and Pawson, T. (1986) Mol. Cell. Biol. 6, 4396–4408.
75 Stahl, M.L., Ferenz, C.R., Kelleher, K.L., Kriz, R.W. and Knopf, J.L. (1988) Nature 332, 269–272.
76 Mayer, B.J., Hamaguchi, M. and Hanafusa, H. (1988) Nature 332, 272–275.
77 Vogel, U.S., Dixon, R.A.F., Schaber, M.D., Diehl, R.E., Marshall, M.S., Scolnick, E.M., Sigal, I.S. and Gibbs, J.B. (1988) Nature 335, 90–93.
78 Rhee, S.G., Suh, P.-G., Ryu, S.-H. and Lee, S.Y. (1989) Science 244, 546–550.
79 Marshall, M.S., Hill, W.S., Ng, A.S., Vogel, U.S., Schaber, M.D., Scolnick, E.M., Dixon, R.A.F., Sigal, I.S. and Gibbs, J.B. (1989) EMBO J. 8, 1105–1110.
80 Verderame, M.F., Kaplan, J.M. and Varmus, H. (1989) J. Virol. 63, 338–348.
81 Mes-Masson, A.M., McLaughlin, J., Daley, G.Q. and Witte, O.N. (1986) Proc. Natl. Acad. Sci. USA 83, 9768–9772.
82 Shtivelman, E., Lifshitz, B., Gale, R.B. and Canaani, E. (1985) Nature 315, 550–554.
83 Rein, A., McClure, M.R., Rice, N.R., Luftig, R.B. and Schultz, A.M. (1986) Proc. Natl. Acad. Sci. USA 83, 7246–7250.
84 Renshaw, M.W., Capozza, A. and Wang, J.Y.J. (1988) Mol. Cell. Biol. 8, 4547–4551.
85 Dickson, C., Eisenman, R. and Fan, H. (1985) in RNA Tumor Viruses (Weiss, R., Teich, N., Varmus, H. and Coffin, J., eds.), pp. 135–146, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
86 Bergold, P.J., Wang, J.Y.J., Hardy, W.D., Littau, V., Johnson, E. and Besmer, P. (1987) Virology 158, 320–329.
87 Prywes, R., Foulkes, J.G., Rosenberg, N. and Baltimore, D. (1983) Cell 34, 569–579.
88 Prywes, R., Hoag, J., Rosenberg, N. and Baltimore, D. (1985) J. Virol. 53, 123–132.
89 Besmer, P., Murphy, J.E., George, F.H., Qui, P.J., Bergold, P.J.,

Lederman, L., Snyder, H.W., Brodeur, D., Zuckerman, E.E. and Hardy, W.D. (1986) Nature 320, 415–421.
90 Hariharan, I.K. and Adams, J.M. (1987) EMBO J. 6, 115–119.
91 Clark, S.S., McLaughlin, J., Timmons, M., Pendergast, A.M., Ben-Neriah, Y., Dow, L.D., Crist, W., Rovera, G., Smith, S.D. and Witte, O.N. (1988) Science 239, 775–777.
92 Fainstein, E., Marcelli, C., Rosner, A., Canaani, E., Gale, R.P., Smith, S.D. and Croce, C.M. (1987) Nature 330, 386–388.
93 Pendergast, A.M., Clark, R., Kawasaki, E.S., McCormick, F.P. and Witte, O.N. (1989) Oncogene 4, 759–766.
94 Pendergast, A.M., Traugh, J.A. and Witte, O.N. (1987) Mol. Cell. Biol. 7, 4280–4289.
95 Watanabe, S.M. and Witte, O.N. (1983) J. Virol. 45, 1028–1036.
96 Murtaugh, K., Skladany, G., Hoag, J. and Rosenberg, N. (1986) J. Virol. 60, 599–606.
97 Rosenberg, N., Clark, D.R. and Witte, O.N. (1980) J. Virol. 36, 766–774.
98 Huebner, R.C., Engelman, A., Schiff, L. and Rosenberg, N. (1988) in B Cell Development (Klineman, N., Witte, O.N. and Howard, M., eds.), pp. 257–270, Alan R. Liss, San Diego, CA.
99 Bernards, A., Paskind, M. and Baltimore, D. (1988) Oncogene 2, 297–304.
100 Bernards, A., Rubin, C.M., Westbrook, C.A., Paskind, M. and Baltimore, D. (1987) Mol. Cell. Biol. 7, 3231–3236.
101 Durcia, D.S., Restrepo, M.A., Thomas, T.L. and Beckingham, K. (1987) Gene 59, 63–76.
102 Dierks, P., Van Ooyen, A., Cochran, M.D., Dobkin, C., Reiser, J. and Weissmann, C. (1983) Cell 32, 695–706.
103 Dynan, W.S. and Tjian, R. (1983) Cell 35, 79–87.
104 Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J.D. (1983) Molecular Biology of the Cell, p. 412, Garland Publishing Company, New York.
105 Muller, R., Slamon, D.J., Tremblay, J.M., Cline, M.J. and Verma, I. (1982) Nature 299, 640–644.
106 Ponzetto, C. and Wolgemuth, D.J. (1985) Mol. Cell. Biol. 5, 1791–1794.
107 Kruh, G.D., King, C.R., Kraus, M.H., Popescu, N.C., Amsbaugh, S.C., McBride, W.O. and Aaronson, S.A. (1986) Science 234, 1545–1548.
108 Hoffman-Falk, H., Einat, P., Shilo, B.-Z. and Hoffman, F.M. (1983) Cell 32, 589–593.
109 Henkenmeyer, M.J., Gertler, F.B., Goodman, W. and Hoffman, F.M. (1987) Cell 51, 821–828.
110 Lev, Z., Leibowitz, N., Segev, O. and Shilo, B.-Z. (1984) Mol. Cell. Biol. 4, 982–984.
111 Telford, J., Burckhardt, J., Butler, B. and Pirrotta, V. (1985) EMBO J. 4, 2609–2615.
112 Gertler, F.B., Bennett, R.L., Clark, M.J. and Hoffman, F.M. (1989) Cell, in press.
113 Nowell, P.C. and Hungerford, D.A. (1960) Science 132, 1497.
114 Rowley, J.D. (1973) Nature 243, 290–293.
115 Lozzio, C.B. and Lozzio, B.B. (1975) Blood 45, 321–334.
116 Rowley, J.D. (1980) Annu. Rev. Genet. 14, 17–39.
117 Propp, S. and Lizzi, F.A. (1970) Blood 36, 353–360.
118 Catovsky, D. (1979) Br. J. Haematol. 42, 493–498.
119 Priest, J.R., Robison, L.L., McKenna, R.W., Lindquist, L.L., Warkenter, P.I., LeBien, T.W., Woods, W.G., Kersey, J.H., Coccia, P.F. and Nesbit, M.E. (1980) Blood 56, 15–22.
120 Heisterkamp, N., Groffen, J., Stephenson, J.R., Spurr, N.K., Goodfellow, P.N., Solomon, E., Carritt, B. and Bodmer, W.F. (1982) Nature 299, 747–750.
121 Bartram, C.R., DeKlein, A., Hagemeijer, Van Agthoven, T., Van Kessel, A.G., Bootsma, D., Grosveld, G., Ferguson-Smith, M.A., Davies, T., Stone, M., Heisterkamp, N., Stephenson, J.R. and Groffen, J. (1983) Nature 306, 277–280.
122 DeKlein, A., Van Kessel, A.G., Grosveld, G., Bartram, C.R., Hagemeijer, A., Bootsma, D., Heisterkamp, N., Groffen, J. and Stephenson, J.R. (1982) Nature 300, 765–767.
123 Gale, R.P. and Canaani, E. (1984) Proc. Natl. Acad. Sci. USA 81, 5648–5652.
124 Canaani, E., Stern-Saltz, D., Aghai, E., Gale, R.P., Berrebi, A. and Jamiszewicz, E. (1984) Lancet i, 593–595.
125 Heisterkamp, N., Stam, K., Groffen, J., DeKlein, A. and Grosveld, G. (1985) Nature 315, 758–761.
126 Heisterkamp, N., Knoppel, E. and Groffen, J. (1988) Nucleic Acids Res. 16, 10069–10081.
127 Heisterkamp, N., Stephenson, J.R., Groffen, J., Hansen, P.F., DeKlein, A., Bartram, C.R. and Grosveld, G. (1983) Nature 306, 239–242.
128 Groffen, J., Stephenson, J.R., Heisterkamp, N., DeKlein, A., Bartram, C.R. and Grosveld, G. (1984) Cell 36, 93–99.
129 Leibowitz, D., Schaefer-Rego, K., Popenoe, D.W., Mears, J.G. and Bank, A. (1985) Blood 66, 243–245.
130 Grosveld, G., Verwoerd, T., Van Agthoven, T., DeKlein, A., Ramachandran, K.L., Heisterkamp, N., Stam, K. and Groffen, J. (1986) Mol. Cell. Biol. 6, 607–616.
131 DeKlein, A., Van Agthoven, T., Groffen, G., Heisterkamp, N., Groffen, J. and Grosveld, G. (1986) Nucleic Acids Res. 14, 7071–7082.
132 Westbrook, C.A., Rubin, C.M., Carrino, J.J., LeBeau, M.M., Bernards, A. and Rowley, J.D. (1988) Blood 71, 697–702.
133 Collins, S.J. and Groudine, M.T. (1983) Proc. Natl. Acad. Sci. USA 80, 4813–4817.
134 Collins, S.J., Kubonishi, I., Miyoshi, I. and Groudine, M.T. (1984) Science 225, 72–74.
135 Rubin, C.M., Carrino, J.J., Dickler, M.N., Leibowitz, D., Smith, S.D. and Westbrook, C.A. (1988) Proc. Natl. Acad. Sci. USA 85, 2795–2799.
136 DeKlein, A., Hagemeyer, A., Bartram, C.R., Hourven, R., Hoefsloot, L., Carbonell, F., Chan, L., Barnett, M., Greaves, M., Kleihauer, E., Heisterkamp, N., Groffen, J. and Grosveld, G. (1986) Blood 68, 1369–1375.
137 Chen, S.J., Chen, Z., Hillion, J., Grausz, D., Loiseau, P., Flandrin, G. and Berger, R. (1989) Blood 73, 1312–1315.
138 Hermans, A., Heisterkamp, N., Von Lindern, M., Van Baal, S., Meyer, D., Van der Plas, D., Wiedemann, L.M., Groffen, J., Bootsma, D. and Grosveld, G. (1987) Cell 51, 33–40.
139 Denny, C.T., Shah, N.P., Ogden, S., Willman, C., McConnell, T., Crist, W., Carroll, A. and Witte, O.N. (1989) Proc. Natl. Acad. Sci. USA, in press.
140 Heisterkamp, N., Jenkins, R., Thibodeau, S., Testa, J.R., Weinberg, K. and Groffen, J. (1989) Blood 73, 1307–1311.
141 Erikson, J., Griffin, C.A., Ar-Rushdi, A., Valtieri, M., Hoxie, J., Finan, J., Emanuel, B.S., Rovera, G., Nowell, P.C. and Croce, C.M. (1986) Proc. Natl. Acad. Sci. USA 83, 1807–1811.
142 Stam, K., Heisterkamp, N., Grosveld, G., DeKlein, A., Verma, R.S., Coleman, M., Dosik, H. and Groffen, J. (1985) N. Engl. J. Med. 313, 1429–1433.
143 Timmons, M.S. and Witte, O.N. (1989) Oncogene 4, in press.
144 Ben-Neriah, Y., Daley, G.Q., Mes-Masson, A.M., Witte, O.N. and Baltimore, D. (1986) Science 233, 212–214.
145 Stam, K., Heisterkamp, N., Reynolds, F.H. and Groffen, J. (1987) Mol. Cell. Biol. 7, 1955–1960.
146 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.
147 Lifshitz, B., Fainstein, E., Marcells, C., Shtivelman, E., Amson, R., Gale, R.P. and Canaani, E. (1988) Oncogene 2, 113–117.
148 Fialkow, P.J., Jacobson, R.J. and Papayannopoulou, T. (1977) Am. J. Med. 63, 125–130.
149 Eaves, A.C., Cashman, J.D., Gaboury, L.A., Kolousek, D.K. and Eaves, C.J. (1986) Proc. Natl. Acad. Sci. USA 83, 5306–5310.

150 Gordon, M.Y., Dowding, C.R., Riley, G.P., Goldman, J.M. and Greaves, M.F. (1987) Proc. Natl. Acad. Sci. USA 83, 5306–5310.
151 Strife, A. and Clarkson, B. (1988) Sem. Hematol. 25, 1–19.
152 Champlin, R.E. and Golde, D.W. (1985) Blood 65, 1039–1047.
153 Maxwell, S.A., Kurzrock, R., Partson, S.J., Talpaz, M., Gallick, G.E., Kloetzer, W.S., Arlinghaus, R.B., Kouttab, N.M., Keating, M.J. and Gutterman, J.V. (1987) Cancer Res. 47, 1731–1739.
154 Huhn, R.D., Posner, M.R., Rayter, S.I., Foulkes, J.G. and Frackelton, A.R. (1987) Proc. Natl. Acad. Sci. USA 84, 4408–4412.
155 Waneck, G.L. and Rosenberg, N. (1981) Cell 26, 79–89.
156 Shtivelman, E., Gale, R.P., Dreazen, O., Berrebi, A., Zaizob, R., Kubonishi, I., Miyoshi, I. and Canaani, E. (1987) Blood 69, 971–973.
157 Konopka, J.B., Clark, S., McLaughlin, J., Nitta, M., Kato, Y., Strife, A., Clarkson, B. and Witte, O.N. (1986) Proc. Natl. Acad. Sci. USA 83, 4049–4052.
158 Bartram, C.R., Janssen, J.W.G., Becher, R., DeKlein, A. and Grosveld, G. (1986) J. Exp. Med. 164, 1389–1396.
159 Rowley, J.D. (1980) Clin. Haematol. 9, 55–86.
160 Janssen, J.W.G., Steenvoorden, A.C.M., Lyons, J., Anger, B., Bohlke, J.U., Bos, J.L., Seliger, H. and Bartram, C.R. (1987) Proc. Natl. Acad. Sci. USA 84, 9228–9232.
161 Hirai, H., Tanaka, S., Azuma, M., Anraku, Y., Kobayoshi, Y., Fujisawa, M., Okabe, T., Urabe, A. and Takaku, F. (1985) Blood 66, 1371–1378.
162 Liu, E., Hjelle, B. and Bishop, J.M. (1988) Proc. Natl. Acad. Sci. USA 85, 1952–1956.
163 Lugo, T.G. and Witte, O.N. (1989) Mol. Cell. Biol. 9, 1263–1270.
164 Kurzock, R., Gutterman, J.U. and Talpaz, M. (1988) N. Engl. J. Med. 319, 990–998.
165 Raport, S.I. (1987) Introduction to Immunology, J.B. Lippincott, Philadelphia.
166 Ribeiro, R.V., Abromowitch, M., Raimondi, S., Murphy, S.B., Behm, F. and Williams, D.L. (1987) Blood 70, 948–953.
167 Catovsky, D. (1979) Br. J. Haematol. 42, 493–498.
168 Tachibana, N., Raimondi, S.C., Laner, S.J., Sartain, P. and Dow, L.W. (1987) Blood 70, 1458–1466.
169 Priest, J.R., Robison, L.L., McKenna, R.W., Lindquist, L.L., Warkentin, P.I., LeBien, T.W., Woods, W.G., Kersey, J.H., Coccia, P.F. and Nesbit, M.E. (1980) Blood 56, 15–22.
170 Kurzrock, R., Shtalrid, M., Talpaz, M., Kloetzer, W.S. and Gutterman, J.U. (1987) Blood 70, 1584–1588.
171 Whang-Pheng, J., Henderson, R.S., Knutsen, T., Freireich, E.J. and Gart, J.J. (1970) Blood 36, 448–457.