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Abl-interactor-1, a novel SH3 protein binding to the carboxy-terminal portion of the Abl protein, suppresses *v-abl* transforming activity

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A novel cellular protein, Abl-interactor-1 (Abi-1), which specifically interacts with the carboxy-terminal region of Abl oncoproteins, has been identified in a mouse leukemia cell line. The protein exhibits sequence similarity to homeotic genes, contains several polyproline stretches, and includes a src homology 3 (SH3) domain at its very carboxyl terminus that is required for binding to Abl proteins. The *abi-1* gene has been mapped to mouse chromosome 2 and is genetically closely linked to the *c-abl* locus. The gene is widely expressed in the mouse, with highest levels of mRNA found in the bone marrow, spleen, brain, and testes. The Abi-1 protein coimmunoprecipitates with v-Abl and serves as a substrate for kinase activity. When overexpressed in NIH-3T3 cells, *abi-1* potently suppresses the transforming activity of Abelson leukemia virus expressing the full-length p160^{v-abl} kinase but does not affect the transforming activity of viruses expressing a truncated p90^{v-abl} or v-src kinases. We suggest that the Abi-1 protein may serve as a regulator of Abl function in transformation or in signal transduction.

[Key Words: Abelson; tyrosine kinase; SH3 domain; leukemia]

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The mammalian *c-abl* gene encodes a nonreceptor tyrosine protein kinase, which is likely to be important in signal transduction pathways during development of lymphoid cells and several other tissues (for recent reviews, see Wang 1993; Kerr 1994; Rosenberg 1994). The gene was originally identified as a homolog of the *v-abl* oncogene carried by the Abelson murine leukemia virus (A-MuLV; Abelson and Rabstein 1970a,b), a potent transforming agent specific for early B lineage cells both in vitro and in vivo (Rosenberg et al. 1975; Alt et al. 1981). The kinase activity of c-Abl is normally tightly regulated, and deregulated transforming alleles can be created by various mutations, including deletions at the amino (Franz et al. 1989; Jackson and Baltimore 1989; Muller et al. 1991) or carboxyl terminus (Bergold et al. 1987; Goga et al. 1993), point mutations (Jackson et al. 1993), fusion to the retroviral *gag* gene in *v-abl* (Goff et al. 1980), or fusion to the breakpoint cluster region (BCR) locus via t(9;22) translocations that create the Philadelphia chromosome, a diagnostic marker found in 95% of cases of chronic myelogenous leukemia and ~10% of acute lymphocytic leukemias in humans (de Klein et al. 1982; Muller et al. 1991; Pendergast et al. 1991). There is

good evidence that in some settings and cells *v-abl* can promote a G₁ → S transition in the cell cycle (Chen and Rosenberg 1992), whereas in others *v-abl* and overexpressed *c-abl* can inhibit cell growth by causing G₁ arrest (Renshaw et al. 1992; Jackson et al. 1993). The pathways by which Abl induces the transformed phenotype remain unclear, although a large number of known partners have been implicated genetically and biochemically in its activity. *V-abl* transformation blocks NF-κB activity in B cells, possibly accounting for its ability to arrest differentiation (Chen et al. 1994; Klug et al. 1994), and dominant negative mutants of *c-myc* can block *v-abl*-mediated transformation, suggesting that *myc* lies downstream of *abl* (Sawyers et al. 1992; Sawyers 1993). V-Abl can phosphorylate the CTD region of RNA polymerase II in vitro (Baskaran et al. 1993), and at least some of its downstream effects may thus be mediated by direct modifications to the transcription machinery.

The c-Abl protein contains a number of recognizable motifs that are likely to serve as docking sites for other partners. The protein is often divided into four regions (for review, see Wang 1993):

1. *Amino-terminal variable region.* Two alternative amino termini for c-Abl proteins, one of which is myristylated, have been identified and shown to arise

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by the splicing of alternative first exons onto the conserved body of the mRNA.

2. *Kinase regulatory regions.* The region near the amino terminus includes *src* homologous SH2 and SH3 domains shared by other tyrosine kinases, PI3 kinase, adaptor proteins Grb2, crk, and nck, GTPase activating protein (GAP), phospholipase C γ , and a number of other proteins (Pawson 1992; Cohen et al. 1995). The SH2 domain binds phosphotyrosine (Mayer et al. 1991) and is believed to play a role in substrate recognition (Koch et al. 1991; Pawson 1992); mutations altering the v-Abl SH2 can block its transforming activity (Mayer et al. 1992) or, more specifically, its B-cell-transforming activity (Hevezi et al. 1992). In contrast, the SH3 domain plays a negative regulatory role in transformation; deletion of the SH3 domain of *c-abl* can activate its transforming activity for NIH-3T3 cells and permit lymphoma induction in mice (Franz et al. 1989; Jackson and Baltimore 1989).
3. *Catalytic protein tyrosine kinase or SH1 domain.* The kinase domain is the most highly conserved structure across the large PTK family. Tyrosine kinase activities are essential for Abl-transforming activity, as for many transforming oncogenes (*src*, *fes*, *erbB*, *fms*), and are assumed to represent essential components of signal transduction pathways that lead to a proliferative response.
4. *Noncatalytic carboxy-terminal domain.* Unlike many other PTKs, Abl proteins have a unique and large (630 amino acids) proline-rich carboxy-terminal region. This domain is much less conserved and appears in only barely recognizable form on the Arg (Abl-related gene product) protein, the closest homolog of Abl (Kruh et al. 1990). The carboxy-terminal region is important for efficient lymphocyte transformation by v-Abl both in vivo and in vitro but dispensable for transformation of NIH-3T3 fibroblasts (Rosenberg et al. 1980; Goff et al. 1981b; Watanabe and Witte 1983). Extensive mutational analysis of this domain and examination of naturally occurring weakly oncogenic viral strains indicate that an intact carboxyl terminus contributes significantly to B-cell tropism (Rosenberg and Witte 1980; Prywes et al. 1983; Hevezi et al. 1992), although it is not absolutely required (Parmar et al. 1991). The carboxyl terminus is also important for normal c-Abl function, as mutant mice homozygous for a substitution mutation truncating the protein show reduced viability and multiple defects in bone, eye, and lymphoid development (Schwartzberg et al. 1991), much like those homozygous for null mutations (Tybulewicz et al. 1991). The presence of these many domains in the carboxyl terminus suggests that c-Abl is normally subject to complex regulation and control.

An approach taken in many laboratories toward understanding the mechanisms of action of the Abl kinase has been the identification of proteins that interact with it. Several proteins that bind to the SH2 and SH3 domains of Abl have been identified (Mayer et al. 1991), including

3BP-1 and 3BP-2 (Cicchetti et al. 1992), but the function of these proteins is unclear (Mayer and Baltimore 1994). Recently the carboxy-terminal portion of the retinoblastoma protein, outside of the A/B pocket, has been shown to bind to the kinase (SH1) domain of Abl and inhibit its kinase activity, providing a potential link to its control of the cell cycle (Welch and Wang 1993). The Abl carboxyl terminus includes the sites for binding to many other proteins. Two distinct regions are recognized by F- and G-actin, accounting for the ability of Bcr-Abl and myristylated forms of c-Abl to associate with F-actin stress fibers in fibroblasts (McWhirter and Wang 1993; Van Etten et al. 1994). Particularly intriguing is the finding that Crk and Nck, two SH2- and SH3-containing adapter proteins, bind to Abl in proline-rich sequences immediately downstream of the kinase domain (Ren et al. 1994). The Crk-1 protein, first identified as the oncogene on the avian sarcoma virus CT10, may transform cells through interaction with Abl proteins (Feller et al. 1994; Nichols et al. 1994). Finally, the carboxy-terminal portion of the Abl proteins have been shown to contain a polylysine nuclear localization signal (NLS) (Van Etten et al. 1989) and a closely linked DNA-binding activity that is controlled by phosphorylation in a cell-cycle-dependent fashion (Kipreos and Wang 1992). The role of this binding in Abl function is unclear.

To further our understanding of the mechanisms of action of the Abl proteins, it will be important to identify cellular factors that regulate its enzymatic activity, the physiologically relevant substrates of Abl tyrosine kinase, and any other proteins that interact with Abl to form active macromolecular complexes. Because the carboxy-terminal region is unique to the Abl group of PTKs and has important roles in both normal lymphopoiesis and lymphoid cell transformation, cellular proteins that interact with this region may be significant partners and, in particular, may act to control or suppress kinase activity. We have identified and characterized one such protein that binds to Abl. The protein, termed Abl-interactor-1 (Abi-1), was found to be a substrate and a potent suppressor of the transforming activity of the activated *v-abl* oncogene, strongly suggesting that this protein can interact with Abl proteins to directly modulate its biological functions. A closely related human protein, Abi-2, is reported also to act as a substrate of the kinase (Dai and Pendergast, this issue), suggesting that a conserved family of SH3 proteins may interact with Abl to regulate its activities.

Results

Identification of an Abl-binding protein, Abi-1

To identify cellular proteins that interact with the Abl carboxy-terminal domain, we made use of the yeast two hybrid system (Fields and Song 1989) to screen a library of fusion proteins. A series of plasmid constructs were generated to express different 3' portions of the Abl protein fused to the LexA DNA-binding domain. One construct, pSHA1000, expressing the region from the poly-

ysine pentapeptide NLS to the Abl DNA-binding domain, was selected for the stability of the protein and its failure to activate reporter β -galactosidase (β -gal) expression by itself (Fig. 1). In this region the c-Abl and v-Abl proteins are identical. A library of plasmid DNAs was generated expressing cDNAs from WEHI-3, a murine macrophage cell line, as fusions to the Gal4 activator domain (Dunaief et al. 1993). Yeast strains containing a suitable *lacZ* indicator gene downstream from a LexA operator were cotransformed to express pairs of hybrid proteins and screened for colonies expressing β -gal. Of 300,000 yeast colonies screened, a total of six independent clones were recovered that stained blue with X-gal; DNA sequence analysis showed that four different genes were represented among them. The gene with the strongest induction of the indicator, represented twice among the clones, was chosen for further analysis and termed *abi-1*.

Several controls were carried out to confirm that the β -gal induction was the direct result of the binding of Abl and Abi-1. The plasmid DNA expressing the protein pGAD-Abi-1 was recovered from yeast and tested in combination with various other DNAs for induction of β -gal (Table 1). The pGAD-Abi-1 DNA scored positive with the LexA-Abl construct, but not with DNA expressing LexA alone (pSH2-1). Abl-interactor-1 failed to interact with irrelevant proteins, including the HIV-1 integrase. In addition, it failed to interact with the closest cellular homolog to Abl, the human Arg protein. These results demonstrate that both the Abl probe region and Abi-1 were required for LacZ induction. Expression of the Abi-1 sequences as a LexA fusion protein resulted in a direct activation of LacZ expression, suggesting that Abi-1 has inherent transcriptional activation functions when artificially tethered to DNA.

cDNA sequence of Abi-1

Sequence analysis of the *abi-1* insert in pGAD-Abi-1, 2.3 kb in length, revealed a single long open reading frame (ORF) fused in-frame to the Gal4 activation domain. To isolate a full-length cDNA, a phage library was screened by hybridization using the *abi-1* insert as probe, and a 2.8-kb cDNA was recovered. The sequence of this clone

Table 1. Yeast two-hybrid tests for the binding of Abi-1 to the Abl carboxy-terminal domain

LexA DNA-binding hybrid ^a (protein moiety/plasmid)	Gal4 transcription activation hybrid ^b (protein moiety/plasmid)	β -Gal activity ^c
Abl carboxy-terminal domain/pSHA100	Abi-1/pGAbi1	++
—	Abi-1/pGAbi1	—
Abl carboxy-terminal domain/pSHA1000	Gal4AC-(null)/pGADNOT	—
LexADB-(null)/pSH2-1	Abi-1/pGAbi1	—
LexADB-(null)/pSH2-1	—	—
Integrase/pSHIN	Abi-1/pGAbi1	—
ARG/pSH-Arg	Abi-1/pGAbi1	—
Abi-1/pSH-Abi1	—	+

β -Gal activity as an indicator of interaction was scored by the intensity of blue color produced by the yeast colonies and time for color development.

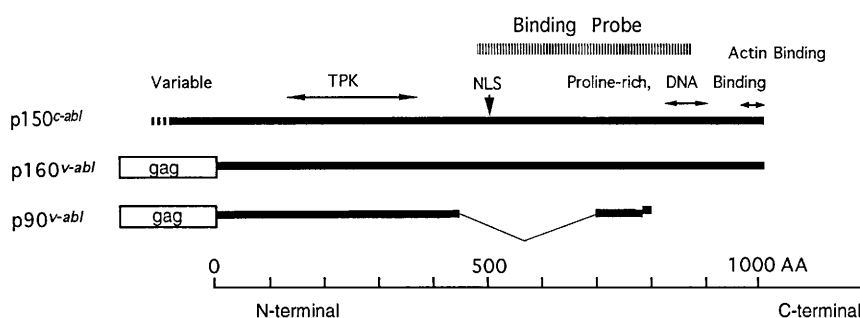
^a(LexADB).

^b(Gal4AC).

^c(+++) Dark blue appeared in <45 min at 37°C; (++) light blue appeared in 4 hr; (—) no blue color in 24 hr.

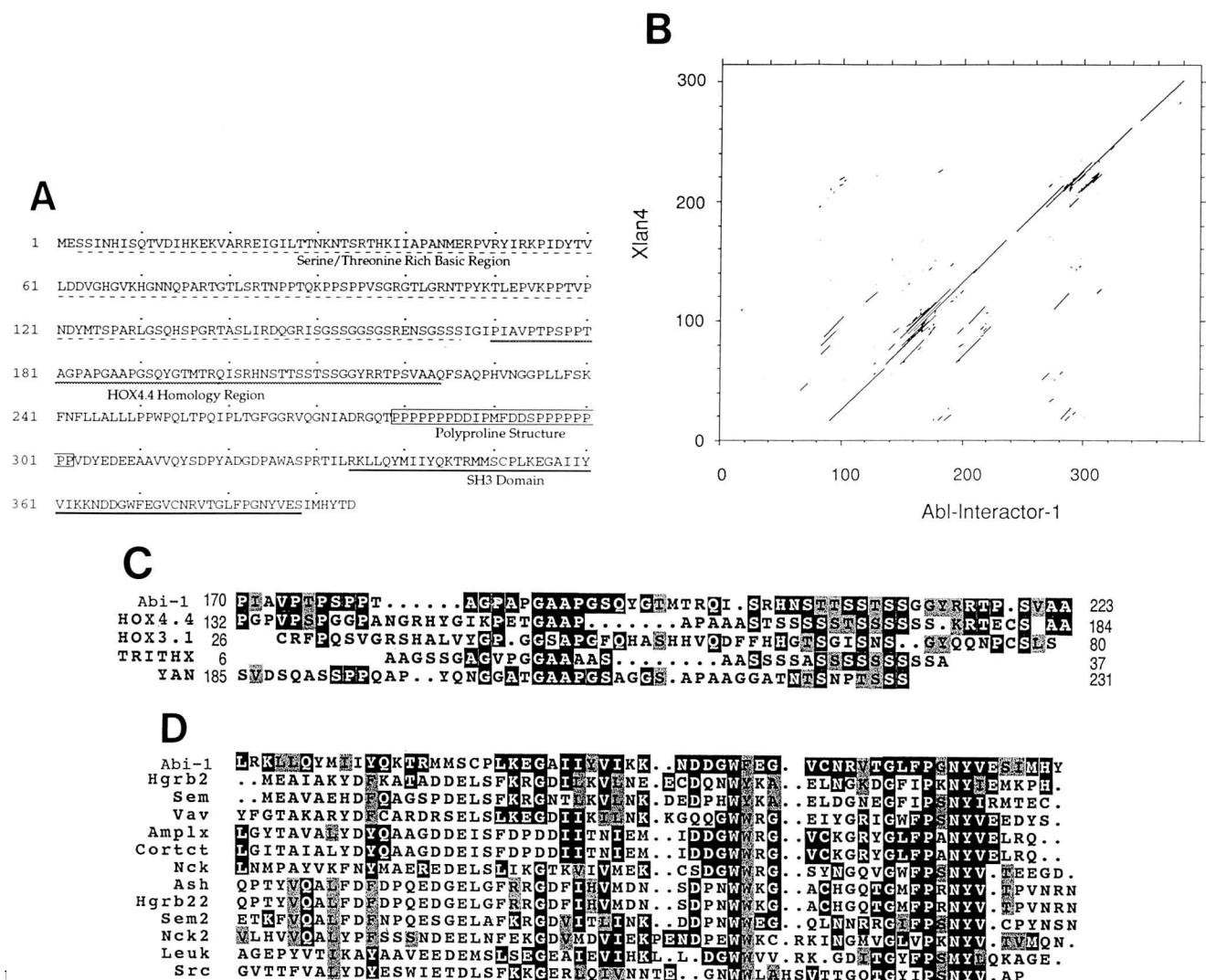
was determined by standard methods (GenBank accession no. MMU 17698) and used to predict the protein sequence (Fig. 2A). An ATG initiation codon near the 5' end of the insert lay in a good match to the canonical eukaryotic translation initiation consensus, suggesting that this may be the start site for translation of the normal mRNA. The frame is open all the way to the 5' end of the clone, however, and thus we cannot rule out the possibility that additional coding sequences lie farther upstream. The sequence predicts the formation of a protein of 394 amino acid residues, with a molecular mass of 42,532 daltons; the protein is basic, with a pI of 10.3. The long 3'-untranslated region of the cDNA contains a polyadenylation signal that is trailed by poly(A).

Comparison among the data base sequences revealed



P90A strains of A-MuLV are shown aligned with c-Abl; the P90A protein contains an internal deletion and a frameshift (displacement) near its carboxyl terminus. Amino acid residues from the Gag/Abl junction in p160^{v-abl} are shown at the bottom.

Figure 1. Structure of c-Abl and v-Abl proteins and binding probe. (Top) The position of the tyrosine protein kinase domain (TPK), the nuclear localization signal (NLS), the proline-rich and DNA-binding domain, and the actin-binding domain. The amino terminus of the protein consists of sequences encoded by either of two alternative exons. The region used in a Gal4 fusion construct (pSHA1000) as a binding probe for the identification of *abi-1* is indicated. The structure of the oncoproteins encoded by the P160 and



two-hybrid clone (GenBank accession no. MMU 17698). Amino acid residues are indicated at left. The *Hox4.4* homology region, the polyproline structure, and the SH3 domain are labeled. (B) Two-dimensional comparison between Abi-1 and Xlan4 proteins. Matches are plotted with GCG compare/dotplot algorithms (stringency 14.0, window size 30). (C) Alignment of the Abi-1 with various genes in the homeo box region. Identities and similarities, respectively, between Abi-1 and each gene: mouse *hox4.4* (Renucci et al. 1992), 39% and 48%; mouse *hox3.1* (Breier et al. 1988), 32% and 36%; *Drosophila trithorax* (Mazo et al. 1990), 35% and 41%; and *Drosophila yan* (Lai and Rubin 1992), 30% and 40%. (D) Alignment of Abi-1 SH3 with related SH3 domains in various genes: human growth factor receptor binding protein (Hgrb2, Lowenstein et al. 1992); *Caenorhabditis elegans* sex muscle determining protein (Sem; Clark et al. 1992); *vav* oncoprotein (Vav; Katzav et al. 1989); *amplexin* (Amplx; Schuurin et al. 1993); cortactin (Cortct; Migliarese et al. 1994); the *nck* homolog of *crk* (Nck; Lehmann et al. 1990); the ASH linker protein (Ash; Matuoka et al. 1992); the second SH3 domains of Hgrb2 (Hgrb22), sem (Sem2), and nck (Nck2); leukemia factor (Leuk; Jackson et al. 1994) and the Src oncoprotein (Src; Schwartz et al. 1983). Identical residues are shaded black; closely related residues are shaded gray. Gaps introduced to improve the alignment are marked by dots.

that the *abi-1* gene and its protein sequence have significant similarity (61% identity at the amino acid level) with a gene identified previously in *Xenopus laevis*, termed *Xlan4* (Reddy et al. 1992). The gene encodes a maternal mRNA transcript localized in the animal pole region of fully grown oocytes. A zygotic counterpart of the mRNA was detected at highest concentrations in the central nerve system in the adult. The similarity between *abi-1* and *Xlan4* extends over the entire ORF, with

the exception of the very amino-terminal portion (~80 amino acids) of mouse Abi-1 (Fig. 2B). In addition, Abi-1 is very similar (65% identical) to Abi-2, a human protein described by Dai and Pendergast (this issue). Other evidence suggests that *abi-1* and *abi-2* are not strict mouse and human homologs of a single gene but, rather, represent two members of a family of related genes present in mammals.

The amino-terminal portion of Abi-1 is very high in

serine/threonine content (23%) and exceedingly basic (calculated $pI=11.6$). The comparison of the amino-terminal portion of Abi-1 to known protein sequences revealed a weak homology to several homeo box proteins, especially Hox4.4, Hox3.1, and trithorax (Fig. 2C). The homologous region has up to 40% similarity. This region is outside of the homeo box domain and only present in Hox4.4, Hox3.1, and trithorax proteins, but the conserved nature may define a signature unique to these three homeotic proteins.

The middle portion of Abi-1 contains several prominent polyproline stretches (Fig. 2A). This structure is typical of the transcription activation domains of many transcription factors, homeotic genes, Epstein-Barr virus nuclear proteins, Wilms' tumor protein, the tumor suppressors p53 and retinoblastoma p105 (*RB*), the enhancer binding protein (C/EBP), the mineralocorticoid receptor, and *c-myc*. The polyproline structures are believed to constitute one class of transcription activation domain. This region may be responsible for the transcriptional activation of Abi-1 detected in yeast when fused to the Gal4 DNA-binding domain. The polyproline structure also includes several copies of the consensus PXXP motif of SH3-binding sites (Ren et al. 1993), suggesting that this part of Abi-1 may bind SH3 domains, either of Abi-1 itself, *c-Abl*, or other proteins. The related Abi-2 protein was isolated on the basis of its binding to Abl SH3 (Dai and Pendergast 1995). Our deletion mutation analysis of this region indicates that it may facilitate Abl-Abi-1 binding (see below). There are also several weak consensus matches to the PEST sequences, sites that destabilize proteins (Rogers et al. 1986; Salama et al. 1994). These sequences suggest that the Abi-1 protein may turn over rapidly.

The very carboxyl terminus of Abi-1 consists of a *src* homology 3 domain (SH3). The SH3 domain of the product of *abi-1* has high similarity to that of *Hgrb2* (growth

factor receptor-binding protein), *vav* (oncogene expressed in hematopoietic systems), *amplaxin* (amplified and overexpressed in breast cancer) and leukemia factor, and only more distant similarity to that of *src* and *abl* (Fig. 2C). SH3 domains, which are frequently present in proteins involved in signal transduction pathways, mediate protein-protein interactions by binding to proline-containing sites (Cicchetti et al. 1992; Pawson 1992; Ren et al. 1993, 1994).

Binding region of Abl-interactor-1

The Abl-binding region of Abi-1 was localized by examination of *abi-1* mutants with successive amino-terminal (*nm* series) and carboxy-terminal (*cm* series) deletions in the yeast two-hybrid system (Fig. 3). The plasmids were used to transform yeast cells harboring pSHA1000, and the Abl-binding activities of these mutants were assayed by X-gal staining of colonies. The carboxy-terminal deletions show that the entire 3'-untranslated region is dispensable (ratio of blue/white colonies >95%), but that removal of the SH3 domain results in complete loss of binding activity (ratio=0%). Thus, the Abi-1 SH3 domain is essential for the Abl-Abi-1 interaction. Examination of the amino-terminal deletions suggested that the homeo box homology region was not important (Fig. 3). Removal of the polyproline domain caused a significant decrease in the LacZ induction; mutant Abi-nm1, containing a bare SH3 domain, binds Abl only weakly, whereas mutants with both polyproline and SH3 domains bind strongly (mutants Abi-nm3, Abi-nm4, Abi-nm5; Fig. 3). In this setting the role of the polyproline stretches is not to serve as an SH3-binding site, because the Abl probe does not contain an SH3 domain; rather, the polyproline region presumably stabilizes the adjacent SH3 structure. These mapping results indicate that the SH3 domain and, to a lesser ex-

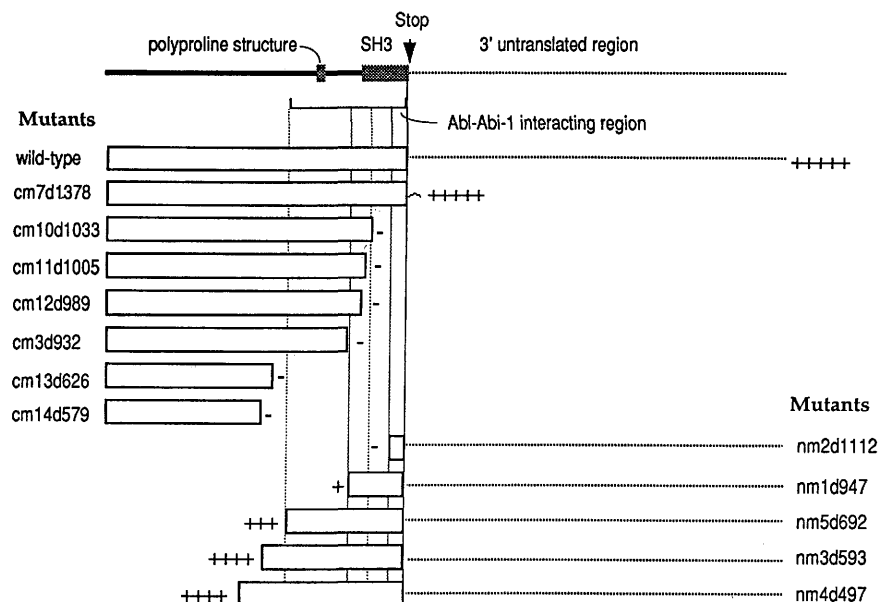


Figure 3. Localization of Abl-binding region of Abi-1 by two-hybrid system. The structure of the parental sequence is indicated at the top; the polyproline and SH3 domains are indicated in the coding region. The structure of carboxy-terminal (*cm*) and amino-terminal (*nm*) deletion mutations of Abi-1 are indicated below. Sequences retained in the constructs are indicated by open bars; the names of the mutants indicate the position of the endpoints in amino acid residues. Mutants fused to the Gal4 activation domain were tested for coactivation with pSHA1000. Interactions with the Abl probe are indicated next to the bars. (++) Strong interaction; (+) weak interaction; (-) no interaction.

tent, the polymeric proline structure of Abi-1 are involved in Abl binding.

Abi-1 expression pattern

The total RNAs from various mouse tissues were analyzed for *abi-1* expression by Northern blot hybridization. RNA samples were extracted from spleen, thymus, testis, brain, heart, muscle, and kidney of young adult mice, as well as NIH-3T3 fibroblast cultures, and the RNA amounts were normalized using β -actin as reference. *Abi-1* mRNA could not be detected in most tissues, and only very low levels were detected in the brain. Two mRNAs, \sim 3.0 and 4.0 kb in size, were observed in the brain (data not shown); the size of the shorter RNA is consistent with the cDNA recovered in pGAD-*Abi-1*, and the origin and structure of the longer mRNA is unclear. An RNase protection assay with a labeled antisense probe was used to test for mRNAs with greater sensitivity. Although the highest level was again found in the brain, *abi-1* mRNA was also detected in testis, bone marrow, spleen, and the myelomonocytic cell line WEHI-3 from which the clone was originally obtained (Fig. 4). Very low levels were found in liver, kidney, and lung, and none could be detected in heart, thymus, muscle, and NIH-3T3 cells. These results suggest that *abi-1* is expressed fairly widely, albeit at low levels. The expression in spleen and bone marrow, and not in thymus, is intriguing and suggests selectivity for B lineage cells, site of A-MuLV-specific tropism for transformation.

abi-1 is closely linked to Abl in the mouse genome

The map position in the mouse genome of a cloned DNA can be readily determined by testing for linkage with polymorphisms among closely related interfertile murine species. We used DNAs from a (C57Bl/6) \times *Mus spretus* F₁ \times *M. spretus* (BSS type) backcross panel to determine the map position of *abi-1*. PCR primers lying in the

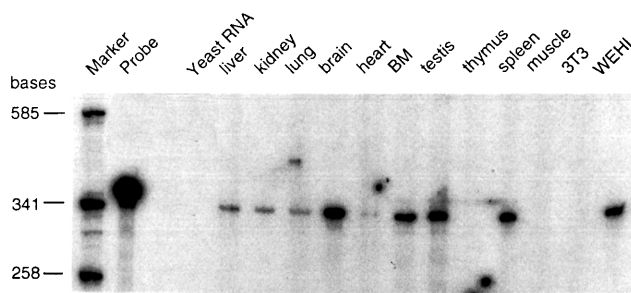


Figure 4. Tissue distribution of expression of *abi-1* mRNA. Total RNAs from the indicated mouse organs or cell lines were used to protect an *abi-1* antisense RNA probe from RNase digestion; products were analyzed by agarose gel electrophoresis and autoradiography. (Marker) Labeled DNA fragments produced by *Sau3A* digest of pUC19 DNA; sizes are indicated at left. (Probe) ³²P-labeled RNA without RNase. (Yeast RNA) Negative control RNA.

3'-untranslated portion of the cDNA were used to amplify a 700-bp DNA fragment from the genomic DNAs of the parental strains. Although the sizes of the amplified DNAs from the two strains were indistinguishable, digestion with *DdeI* generated four products, with a restriction fragment length polymorphism (RFLP) in the smallest ones (60-bp doublet in *M. musculus* vs. 75 and 60 bp in *M. spretus*). The alleles were then characterized in each of 94 DNA samples in the backcross panel obtained from the Jackson Laboratory (Bar Harbor, ME; Rowe et al. 1994). Haplotype analysis from these mapping data indicated that the *abi-1* locus is positioned between *Jund2* and *D2Bir5* (Birkenmeier anonymous DNA fragment 5) markers on mouse chromosome 2 (Fig. 5A). One of the most closely linked biological markers is *c-abl*, \sim 5 cM telomeric to *abi-1* (Fig. 5B). As predicted by synteny, the map position of *abi-1* corresponds to human chromosome 9q32-34; the locus in humans would be telomeric to *c-abl*. Thus, the t(9;22) translocations forming the Philadelphia chromosome would result in the cotranslocation of *abi-1* with *c-abl* to chromosome 22.

Binding and phosphorylation of Abi-1 by Abl proteins in vitro

To determine whether the activation in yeast reflected a direct interaction between the Abl and Abi-1 proteins, *in vitro* binding experiments were performed. The Abi-1 sequence was inserted into an expression vector to direct the synthesis of a recombinant glutathione S-transferase (GST) fusion protein in bacteria. The GST-*Abi-1* fusion was purified from bacterial lysates by binding to glutathione-agarose beads. The beads containing the isolated protein were incubated with cell extracts prepared from cells transformed by A-MuLV strains P160 (encoding p160^{v-abl}) or strain P90A (encoding the shorter p90^{v-abl} protein), or from untransformed NIH-3T3 cells, and then washed extensively with the binding buffer. The bound materials were eluted, resolved by electrophoresis on SDS-polyacrylamide gels, and immunoblotted with anti-Abl antibodies. Although the control GST protein alone did not bind any proteins reactive to anti-Abl antibodies, GST-*Abi-1* bound high levels of p160^{v-abl} in A-MuLV-transformed cells (Fig. 6A). In contrast, GST-*Abi-1* did not bind the closely related v-Abl-transforming protein of the P90A strain, p90^{v-abl} (Fig. 6B). The lack of interaction with p90^{v-abl}, a naturally occurring mutant lacking most of the carboxy-terminal portion of p160^{v-abl} or p150^{c-abl} (Fig. 1; Goff et al. 1981b; Murtagh et al. 1986), is consistent with the requirement for the Abl carboxyl terminus for binding in yeast. In these experiments, the levels of v-Abl proteins swamp out c-Abl and preclude its detection. To test for interaction with c-Abl, lysates from uninfected NIH-3T3 cells were exposed to GST-*Abi-1* beads. Even though the p150^{c-abl} protein is present at very low levels as compared with v-Abl proteins, binding could be observed (Fig. 6C). The largest band detected with the anti-Abl antibody, migrating at 150 kD, comigrates with c-Abl; low molecular mass bands detected in these experiments are degradation

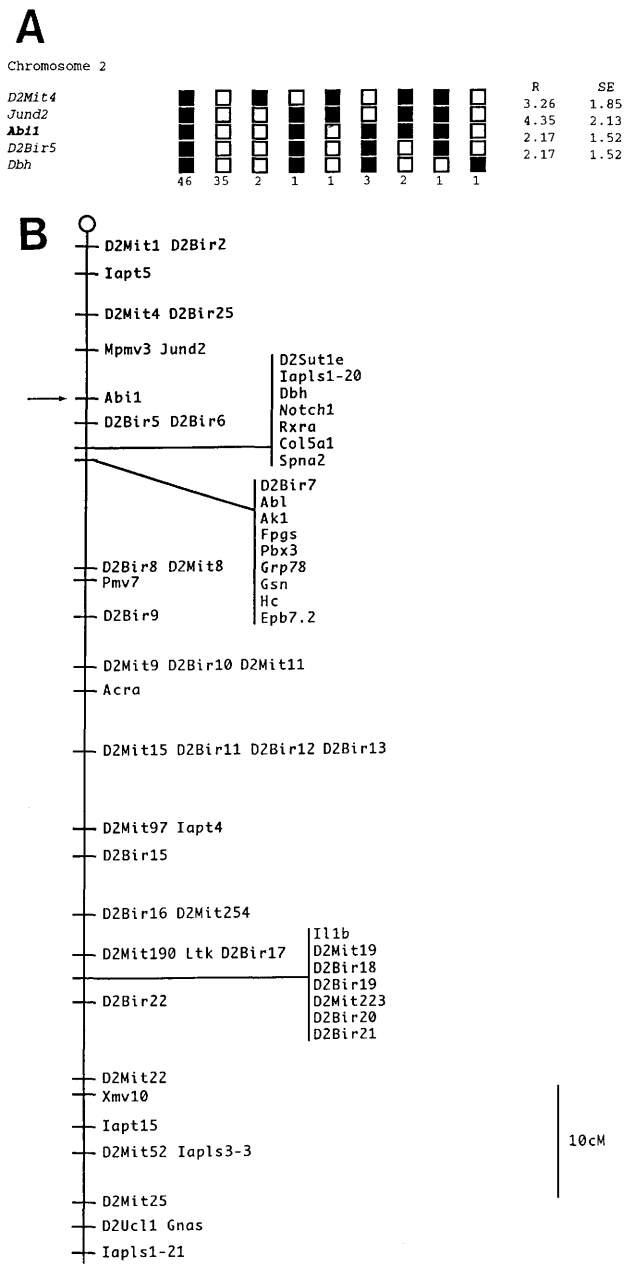


Figure 5. Mapping of the *abi-1* gene to chromosome 2. (A) Haplotype analysis of chromosome 2 markers in backcross mice from (B6×*M. spretus*) F₁ × *M. spretus* backcross (BSS type) showing linkage and relative position of *abi-1*. (■) Inheritance of the C57BL/6j allele; (□) the *M. spretus* allele. Gene names and loci are from GBASE. The number of recombinants are listed below each column, and the recombination frequency between adjacent loci (R) and standard error (SE) are indicated at right. (B) Position of *abi-1* on chromosome 2, just centromeric to *c-abl*.

products of c-Abl and other proteins detected by this antibody, apparent only after the long development times required for c-Abl detection. These results suggest that both full-length v-Abl and c-Abl proteins are in a suitable conformation to bind to Abi-1. The interaction of Abi-1 and Abl was independent of divalent ions such

as Mg²⁺ and Ca²⁺, neither the addition of 20 mM Mg²⁺ and Ca²⁺, nor addition of EDTA and EGTA, had any effect on the binding activity.

To test whether bound Abi-1 could serve as a substrate for the v-Abl kinase after binding *in vitro*, beads containing various protein complexes were incubated with [³²P]ATP and the products were then analyzed by electrophoresis and autoradiography. Beads containing GST-Abi-1 and complexed with the P160^{v-abl} kinase showed strong phosphorylation of the GST-Abi-1 protein, suggesting that bound Abi-1 was a substrate (Fig. 6D). Trace autophosphorylation of the p160^{v-abl} could also be detected. The amount of label detected in the experiments was very high as compared with phosphorylation of other proteins by similar amounts of v-Abl, including autophosphorylation of v-Abl. Beads containing GST-Abi-1 and exposed to buffer alone, or lysates of NIH-3T3 cells or P90A-transformed NIH-3T3 cells did not show phosphorylation of Abi-1. Beads containing GST alone bound no detectable kinase activity. These results demonstrate that Abi-1 bound to v-Abl *in vitro* was properly positioned to serve as a substrate for the kinase. Additional experiments, in which purified GST-Abi-1 protein was added to immunoprecipitated p160^{v-abl}, did not show any inhibition of overall autokinase activity (data not shown). These results suggest that Abi-1 is not a direct inhibitor of the v-Abl kinase activity *per se*.

Abi-1 suppresses v-Abl-transforming activity

To determine the effect of Abi-1 on the transforming activity of v-Abl oncoproteins, we measured the focus-forming activities of Abelson leukemia viruses on NIH-3T3 cell lines induced to overexpress Abi-1 protein. The Abi-1 sequence was inserted downstream of the cytomegalovirus (CMV) early promoter to form plasmid pCGN-Abi-1, and NIH-3T3 cell lines were cotransformed with a mixture of this expression plasmid and the pWLneo plasmid encoding neomycin resistance. G418 resistant clones (G418^r) were picked, expanded, and tested for expression of Abi-1 protein by immunoblotting proteins in extracts using an Abi-1-specific antiserum (Fig. 7A). Of 14 randomly picked clones, 3 were found to overexpress Abi-1 (G418^r/Abi⁺); this efficiency of cotransformation for unlinked DNAs is typical and does not suggest significant toxicity for the gene. No detectable alterations of cell morphology, doubling time, or contact inhibition upon reaching confluence were seen in the Abi-1-overexpressing cells. The expressing and nonexpressing clones were all analyzed in parallel to provide controls.

Virus preparations of two strains of Abelson leukemia viruses P160 and p90A that encode p160^{v-abl} and p90^{v-abl} oncoproteins, respectively, were tested for their transforming activities in those cell lines. The viral stocks were prepared by rescuing Abelson viruses from nonproducer-transformed NIH-3T3 cell lines with Moloney murine leukemia virus (Mo-MuLV) as helper. Tenfold serial dilutions of filtrates from the viral cultures were inoculated onto all 14 G418^r cells and their parental

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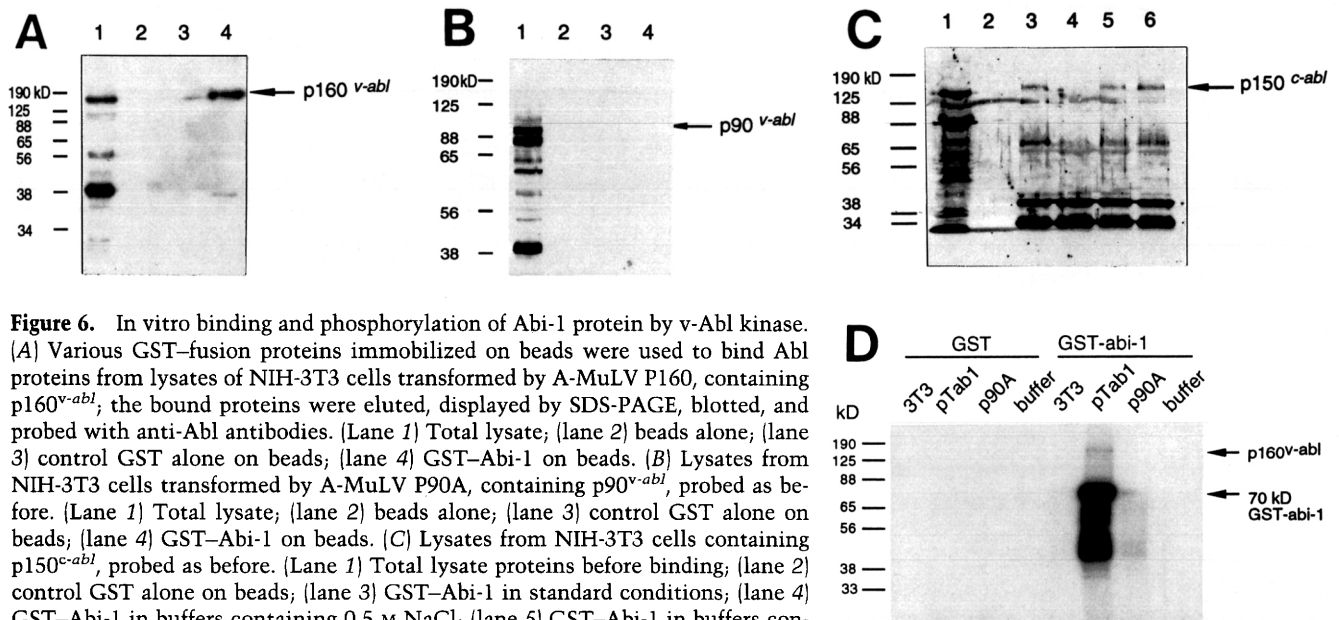


Figure 6. In vitro binding and phosphorylation of Abi-1 protein by v-Abl kinase. (A) Various GST–fusion proteins immobilized on beads were used to bind Abl proteins from lysates of NIH-3T3 cells transformed by A-MuLV P160, containing p160^{v-abl}; the bound proteins were eluted, displayed by SDS-PAGE, blotted, and probed with anti-Abl antibodies. (Lane 1) Total lysate; (lane 2) beads alone; (lane 3) control GST alone on beads; (lane 4) GST–Abi-1 on beads. (B) Lysates from NIH-3T3 cells transformed by A-MuLV P90A, containing p90^{v-abl}, probed as before. (Lane 1) Total lysate; (lane 2) beads alone; (lane 3) control GST alone on beads; (lane 4) GST–Abi-1 on beads. (C) Lysates from NIH-3T3 cells containing p150^{c-abl}, probed as before. (Lane 1) Total lysate proteins before binding; (lane 2) control GST alone on beads; (lane 3) GST–Abi-1 in standard conditions; (lane 4) GST–Abi-1 in buffers containing 0.5 M NaCl; (lane 5) GST–Abi-1 in buffers containing additional 20 mM MgCl₂ and CaCl₂; (lane 6) GST–Abi-1 with additional 20 mM EDTA and EGTA. (D) In vitro phosphorylation of GST–Abi-1 by bound v-Abl kinase. GST (left) or GST–Abi-1 (right) was used to bind proteins from lysates of the indicated cell lines. Bound complexes were incubated with [γ -³²P]ATP, eluted, and analyzed by electrophoresis and autoradiography. Position of migration of marker proteins are indicated at left. The labeled GST–Abi-1 and autophosphorylated p160^{v-abl} are indicated at right.

NIH-3T3 cell control in a standard focus-forming assay. Infected cultures were allowed to grow to confluence, and transformed foci were counted after 12 days (Fig. 7B). The P160 virus typically exhibited titers of 10⁴ FFU/ml on NIH-3T3 cells and G418^r/Abi-1⁻ cells. The three G418^r/Abi-1⁺ cell lines, however, showed strong inhibition of transformation activity, producing significantly fewer foci: 10- to 100-fold fewer than the parental NIH-3T3 cells or the control G418^r/Abi-1⁻ cells. The perfect correlation of Abi-1 overexpression with the greatly reduced focus-forming activity strongly suggests the involvement of Abi-1 in the transforming activity of the P160 strain. In contrast, there was no significant change of the P90A strain's focus-forming activity in the G418^r/Abi-1⁺ cell lines (Fig. 7C). Thus, the suppression of transformation by *abi-1* requires the carboxy-terminal portion of the v-Abl protein. The overexpression of *abi-1* did not block the general ability of the cells to become transformed by all v-Abl proteins, suggesting that the loss of p160^{v-abl}-transforming activity did not result from the loss of a fundamental signal response pathway.

The observed loss of transforming activity in G418^r/Abi-1⁺ cell lines could, in principle, be accounted for by a block to virus replication. To test whether the cells were infected with virus, supernatant culture medium from the G418^r and G418^r/Abi-1⁺ cell lines after infection with the P160 virus were collected. The level of helper virus was assessed by measuring the reverse transcriptase (RT) enzyme activity in the supernatant. RT activities from the G418^r/Abi-1⁻ or G418^r/Abi-1⁺ cells did not reveal any decrease in viral replication compared with that of the parental cell line (data not shown). Thus, we can attribute the reduced P160 viral-transforming ac-

tivity in G418^r/Abi-1⁺ cells to the specific suppression of p160^{v-abl} function instead of decreased viral replication. In principle, the lack of transformed cells could be attributable to genuine transformation resistance or to inappropriate cell death upon infection; the latter possibility is not implausible, given that v-abl expression can be toxic in some settings (Zeigler et al. 1981). To test whether the A-MuLV genome was successfully integrated, expressed, and maintained in the lines, viral supernatants were collected and the focus-forming titers present in these supernatants were determined by infection of fresh NIH-3T3 cells. The Abelson viruses produced by the G418^r/Abi-1⁺ cell lines retained full transforming potency; no significant difference in titer was found in comparing virus released from the parental NIH-3T3 cells or from the G418^r/Abi-1⁻ cells (range 1 × 10² to 5 × 10² FFU/ml; data not shown). Therefore, Abi-1 did not in some way alter the P160 virus or the v-abl gene, block production of A-MuLV RNA or release of virion particles, or sensitize the cells to v-abl toxicity.

The effect of Abi-1 on NIH-3T3 cell transformation by v-src was also assessed. Src and Abl belong to the same protein tyrosine kinase family and share substantial similarity in their SH2, SH3, and kinase catalytic domains. Both v-src and v-abl transform NIH-3T3 cells, although subtle morphological differences are apparent between their transformed cells and foci. A virus carrying the v-src oncogene (Hevezi et al. 1993), using Mo-MuLV as helper, was similarly titered on the G418^r/Abi-1⁺ and G418^r/Abi-1⁻ cell lines for its focus-forming activity. No appreciable difference in the v-src-transforming activity was observed in Abi-1⁺ versus Abi-1⁻ cells (Fig. 7D). Thus, Abi-1 did not act on unrelated tyrosine ki-

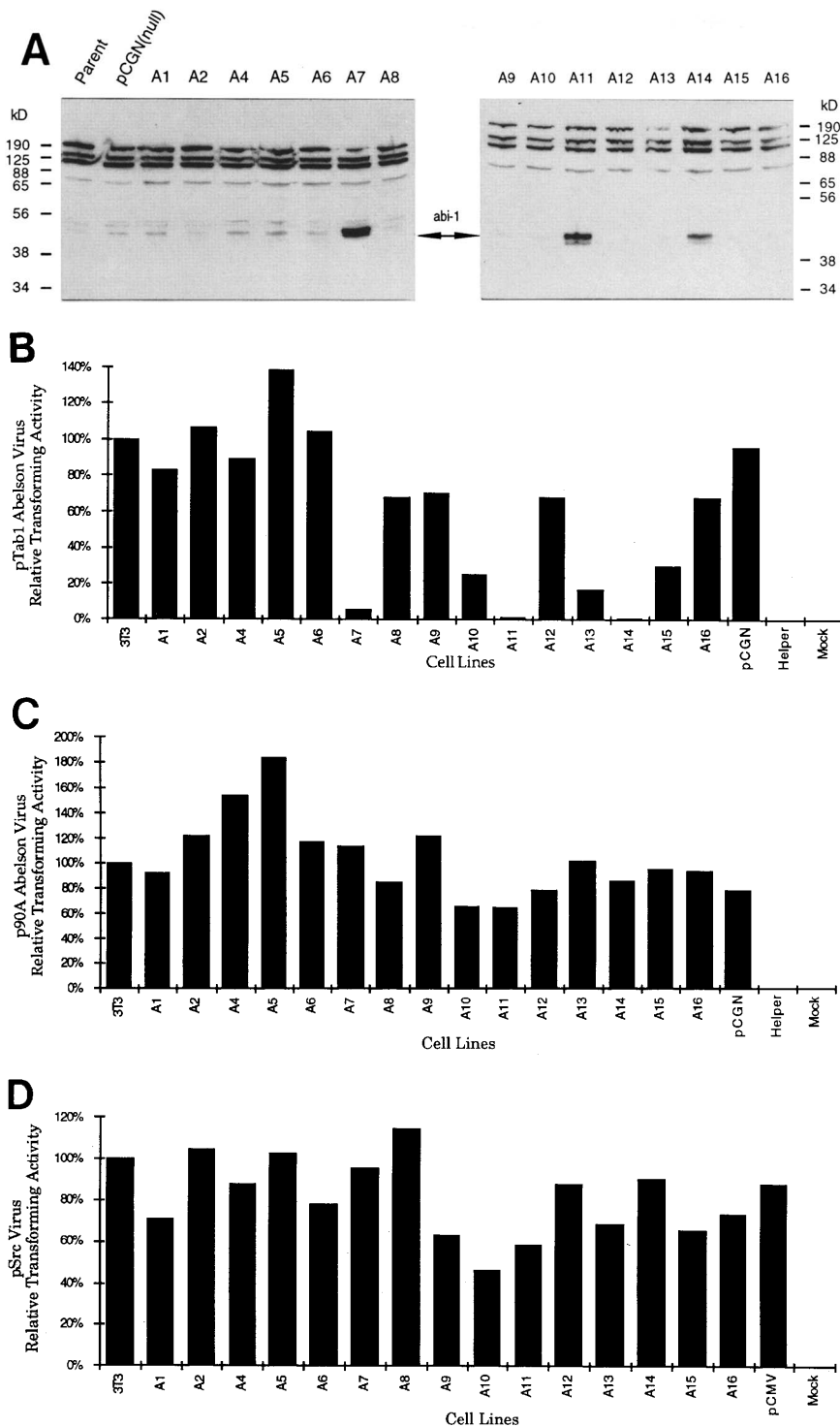


Figure 7. Generation and analysis of NIH-3T3 clones overexpressing the Abi-1 protein. (A) Western blot of proteins from cloned cell lines. After transfection with a mixture of DNAs encoding Abi-1 and neomycin resistance, individual G418^r clones were picked and expanded, extracts were prepared, and proteins were blotted and probed with polyclonal antibodies raised against the GST-Abi-1 protein (lanes A1–A16). Extracts from NIH-3T3 cells (parent) and from a clone transfected with the *neo* gene alone [pCGN(null)] are also shown. Several lines overexpressing the 45-kD Abi-1 protein were identified. (B) Transforming efficiency of the P160 strain of A-MuLV on individual cell lines. The titers of virus (in FFU/ml) are indicated for each host cell line relative to the titer on the parental NIH-3T3. 100% = 4.7×10^4 FFU/ml. (pCGN) Cells transformed only for *neo*^r; (Helper) NIH-3T3 cells infected with M-MuLV helper virus alone; (Mock) NIH-3T3 cells. (C) Transforming efficiency of the P90A strain of A-MuLV on individual cell lines. 100% = 6.5×10^4 FFU/ml. (D) Transforming efficiency of Moloney-based vector carrying the *v-src* oncogene. 100% = 4.1×10^4 FFU/ml.

nases to block the downstream effects of elevated kinase levels.

Coimmunoprecipitation of Abi-1 and v-Abl proteins

To determine whether Abi-1 protein was associated with Abl proteins *in vivo*, attempts were made to coimmuno-

precipitate a complex from cell lysates. Cells expressing both proteins were generated by acute infection of the Abi-1-positive clone A7 cells with either the P160 or P90A strain of A-MuLV. At 48 hr postinfection, lysates were prepared and the Abi-1 protein was immunoprecipitated with a polyclonal anti-Abi-1 antiserum raised against GST-Abi-1 in rabbits. The proteins were col-

lected on protein A-agarose beads, and the bound proteins were eluted, separated by SDS-PAGE, and blotted to nitrocellulose. The blots were then probed with anti-Abl monoclonal antibodies. As a control the v-Abl proteins were also directly immunoprecipitated with anti-Abl sera and analyzed on the same blots. The results showed that the anti-Abi-1 antiserum, but not preimmune serum, efficiently coprecipitated the p160^{v-abl} protein (Fig. 8), nearly as well as the anti-Abl antibody. In contrast, the anti-Abi-1 antiserum did not bring down the truncated p90^{v-abl} protein (Fig. 8). Thus, the majority of the v-Abl protein of the P160 strain is present in complex with Abi-1. The formation of the complex correlated with the presence of the carboxy-terminal sequences of v-Abl, and with the specific suppression of v-Abl-transforming activity by Abi-1, suggesting that the activity was mediated by direct binding of the two proteins in vivo.

Discussion

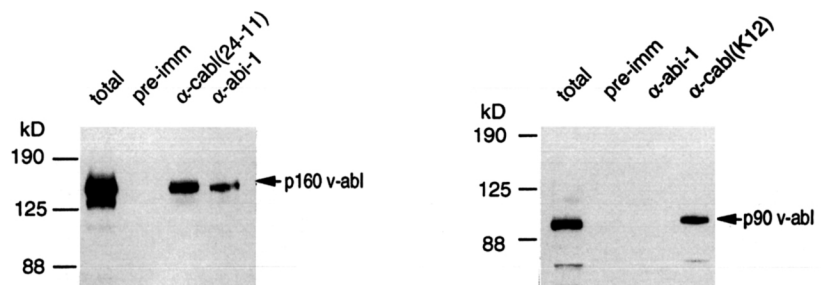
The carboxy-terminal domain of Abl has been of particular interest as a unique structure of the tyrosine kinases. This structure may have roles in the specific functions associated with Abl proteins: tropism for transformation of pre-B lymphoid cells (Rosenberg and Witte 1980; Parmar et al. 1991), cytotoxicity in cultured cells (Zeigler et al. 1981), and the various phenotypes displayed by c-Abl carboxy-terminal domain knockout mice (Schwartzberg et al. 1991). The carboxy-terminal region may thus help to specify the various targets of the Abl kinase. The ability of Abi-1 to bind specifically to this part of Abl proteins and block v-Abl transformation suggests that it plays a negative role in Abl kinase function or target selection. Abi-1, however, does not seem to inhibit the overall kinase activity of v-Abl. The simplest model for the mode of action of Abi-1, when highly overexpressed, is as an inhibitor of the kinase's ability to bind to other critical targets. Abi-1 binding could act by competition to interfere with the localization, folding, or binding of the kinase to such targets. The fact that the inhibitory activity absolutely requires the carboxy-terminal interaction domain of v-Abl strongly suggests that the mechanism occurs via the direct binding to v-Abl rather than through some downstream step in the pathway. The fact that deletions of the carboxyl terminus of v-Abl can enhance the transforming activity of viral vari-

ants (Rosenberg and Witte 1980) is consistent with a negative role for Abi-1 binding to this region in the wild-type protein. Abi-1 bound to both v-Abl and c-Abl in vitro (Fig. 6), and it may play a role in signaling for both proteins. The functions of c-Abl remain unclear, however, and a determination of Abi-1's functional relationship with c-Abl must await new readouts of its activities. It should be emphasized that suppression activity was only observed in a setting of Abi-1 overexpression and that at lower concentrations the normal functions of Abi-1 could serve either as a negative or positive regulator of Abl function.

The reduction in the number of foci seen with the P160 strain of A-MuLV, as opposed to the complete elimination of foci, may reflect the relative level of expression of v-abl and abi-1. Individual clones of cells infected by A-MuLV exhibit a wide range of levels of expression of the viral oncoprotein, presumably reflecting position effects of the different integration sites of the proviral DNA. If a threshold level of Abi-1 protein is required to block transformation, a few clones would be generated by infection that would express high enough levels of v-Abl to overcome the block. Such clones could account for the residual transformants.

The product of the newly identified gene *abi-1* joins a large number of SH3-containing proteins, including most prominently the tyrosine kinases (such as Src, Abl, and receptor kinases) and the adaptor proteins (such as Grb2, Sos, and Crk), that may play roles in signal transduction (Koch et al. 1991; Pawson 1992; Cohen et al. 1995). The proteins binding to Abl at the most similar position as Abi-1 are the products of the *crk* oncogene and its relative *nck* (Ren et al. 1994). Although the binding sites for these proteins are not identical to that for Abi-1 (Fig. 1), they are close enough in the primary sequence that Abi-1 might well interfere with their binding or compete with them for access to Abl. SH3 domains are thought to mediate the binding of the various signal transduction proteins to polyproline motifs on a variety of target proteins (Ren et al. 1993). Abi-1 and its family members may thus be bound to polyproline stretches on a number of such other targets. However, it should be noted that Abi-1 also includes a polyproline stretch of its own, and, in particular, proline motifs common to SH3-binding sites. Its SH3 domain may thus bind intramolecularly to its own proline-rich region or might promote head-to-tail homodimerization with another

Figure 8. Coimmunoprecipitation of v-Abl proteins with Abi-1. V-Abl proteins were immunoprecipitated from lysates of P160 (left) or P90A (right)-transformed NIH-3T3 cells with the indicated antibody preparations. The precipitated proteins were displayed by electrophoresis, blotted, and probed with Abl-specific monoclonal antibody 24-11 (left) or K12 (right). The positions of marker proteins are indicated at left; those of v-Abl proteins at right.



Abi-1 molecule. Furthermore, the proline region may bind to other SH3 domains. The proline-rich region of the closely related Abi-2 protein binds specifically to the SH3 domain of c-Abl (Dai and Pendergast, this issue). Abi-1 does not contain any SH2 motifs, and it is not clear whether it serves to bridge Abl to any other proteins.

The homeo box similarity in the central portion of the molecule may indicate that Abi-1 is bound to DNA, but direct DNA-binding activity has not been observed. The most similar gene in the data base is *xlan4*, a gene of *Xenopus* expressed as a maternal mRNA accumulated in the oocyte (Reddy et al. 1992). A zygotic cognate of that mRNA is also expressed during the formation of the developing central nerve system; in situ hybridization suggests that the gene is expressed in the forebrain, mid-brain, hindbrain, and spinal cord. Although it is intriguing that Abi-1 is also expressed in the brain, there is no evidence of a function for c-Abl in neural development. The similarity to a human gene, *Abi-2*, whose product also binds to Abl proteins as described in Dai and Pendergast (this issue), suggests that the gene and the interaction are both conserved among mammals. The rather limited sequence similarity between these two genes (for mouse vs. human) suggests that they are not strict homologs but simply related members of a multigene family present in both mouse and humans.

The *abi-1* gene maps to mouse chromosome 2 near *c-abl*. It is unclear whether this linkage reflects any functional relationship. Based on synteny, this corresponds to human chromosome 9q32–34, telomeric to human *c-abl*. Thus, the *abi-1* gene is likely cotranslocated with human chromosome 22 during the formation of the Philadelphia chromosome. Given the *abi-1* transformation-suppressing activity and its proximity to the major cytogenetic alteration, it is possible that *abi-1*—or perhaps its loss of expression—plays a role in the pathogenesis in chronic myelogenous leukemia (CML). By examining *abi-1* expression levels or other changes in Ph⁺ leukemia cell lines or patients, a correlation may be obtained for the prediction of the progression or the prognosis of leukemia. There have been reports that homeotic genes may be involved in certain types of cancers. For example, deletions spanning the *Hox4.1* locus were found in a majority cases of a panel of mouse myeloid leukemias (Blatt and Sachs 1988); and translocations involving *MLL*, a mammalian gene with similarity to the *Drosophila* trithorax gene, are frequently found in several acute lymphoblastic and myeloid leukemias in humans (Rowley 1993).

The fact that the Abi-1 protein can act genetically as a tumor suppressor with specificity for the Abl protein suggests that overexpression of the protein could in principle serve to block tumor formation or progression in CML patients bearing the Philadelphia chromosome. Thus, constructs overexpressing the protein might find therapeutic applications. Successful uses await technology for efficient gene transfer of expression constructs into tumor cells and tissues. The identification of *abi-1* as a modifier of *abl* function may provide new insights into the mechanisms by which c-Abl kinase transmits

signals to control target gene expression, and by which v-Abl mishandles that process.

Materials and methods

Yeast two-hybrid system for identifying interacting proteins

cDNA library from the murine myeloid cell line WEHI-3 (Stratagene) was transferred into the yeast expression vector pGAD-NOT, and clones were collected in pools of 100,000 each (Luban et al. 1992; Dunaief et al. 1993). A fragment of the coding region of the v-Abl carboxy-terminal domain (nucleotides 3237–4366; Hevezi et al. 1992) was inserted into yeast expression vector pSH2-1 (Hanes and Brent 1989) to form pSHA1000, encoding a LexA DNA-binding Abl hybrid. Hybrid protein expression from this construct was verified by Western blot with anti-Abl antibody. Plasmids were used to transform yeast strain CTY10-5d (gift of R. Sternglanz, State University of New York, Stony Brook) by the LiAc-PEG method as described previously (Ausubel et al. 1987). Double transformants were plated on Leu⁻His⁻ selection plates and tested for interacting two-hybrid clones by staining nitrocellulose replicate lifts with X-gal as described previously (Kalpana et al. 1994).

DNA cloning, sequencing, and data analysis

DNA manipulations were by standard methods (Maniatis et al. 1982). DNA sequencing was performed with plasmid templates on an automated BioSequencer (Applied Biosystems). The sequence data were analyzed by BLAST comparisons (National Library of Medicine) or GCG sequence analysis software package version 7 (Genetics Computer Group, Inc.).

Deletion mutagenesis and binding domain mapping

Unidirectional deletions were introduced into the *abi-1* cDNA on plasmid pGADNOT by nested exonuclease III/S1 treatment at room temperature according to specifications of the manufacturer (Promega). Clones were picked after transformation of the mutagenized DNAs derived from various times of exonuclease treatment. Clones were sequenced and tested for their interaction with Abl protein in the yeast two-hybrid system.

Expression and purification of recombinant GST-fusion proteins

Recombinant GST-fusion proteins were produced by plasmids based on pGEX2TK (Pharmacia). The production and purification of the fusion proteins followed the manufacturer's specifications. Briefly, *Escherichia coli* cells harboring the plasmid in mid-log phase were induced with 1 mM IPTG. The cells were disrupted by freeze-thaw and sonication in the presence of 1 mM PMSF. The GST-fusion protein was affinity purified by glutathione-agarose beads (Sigma) from a clarified cell lysate. The amount and homogeneity of the products were analyzed by electrophoresis on SDS-polyacrylamide gels stained by Coomassie blue R250.

Binding and phosphorylation of GST-Abi-1 by v-Abl proteins in vitro

Cell extracts were prepared from NIH-3T3 cells, or cell lines transformed by either P160 or P90A strains of A-MuLV. Cells were washed gently three times with chilled PBS supplemented with 50 mM glucose. The cells were then resuspended in TNEN buffer (50 mM Tris-HCl at pH 7.5, 50 mM NaCl, 2 mM EDTA,

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0.5% NP-40, 1 mM PMSF, 2 μ g/ml each of pepstatin, leupeptin, and aprotinin) at $\sim 10^7$ cells/ml and lysed by sonication. The lysate was clarified by centrifugation at 10,000g. The total protein concentration was typically 5 mg/ml. In standard Abl–Abi-1-binding assays, 400 μ l of cell extract was agitated gently on ice for 15 min with 10 μ l glutathione–agarose slurry charged with GST–Abi-1 fusion protein. The unbound fraction was removed by washing the beads with the TNEN buffer. The bound materials were resolved by SDS-PAGE and immunoblotted with specific antibodies. To monitor kinase activity, the beads and their bound proteins were washed as described previously and added to 100 μ l of kinase buffer (50 mM Tris-HCl at pH 8.0, 50 mM NaCl, 6 mM MnCl_2 , 1 mM CTP, 0.2 mM Na_3VO_3 , 1 mM PMSF, 2 μ g/ml each of pepstatin, leupeptin, and aprotinin). The reaction was initiated by addition of 20 μ Ci of [γ - ^{32}P]ATP (5000 Ci/mmol), allowed to proceed for 30 min at room temperature, and terminated by repeated washing of the beads with kinase buffer. The bound proteins were eluted by boiling in 40 μ l of sample buffer for 2 min, and 1 μ l of the eluate was analyzed by electrophoresis on 12% polyacrylamide gels followed by autoradiography.

Coimmunoprecipitation of Abi-1 and Abl proteins

The A7 clone of NIH-3T3 cells expressing Abi-1 protein was infected with the P160 or P90A strain of A-MuLV by coculture with appropriate virus-producing cell lines; $\sim 5 \times 10^6$ cells of each line were mixed and plated together with a $2.5 \times$ dilution. After 48 hr, the cultures were lysed with 2 ml of TNEN buffer, and the lysate was clarified by centrifugation. Lysate (400 μ l) was incubated with anti-Abi-1 antiserum for 1 hr, and the immunocomplexes were collected on protein A–agarose beads. The bound material was eluted in sample buffer and analyzed by electrophoresis on a 10% polyacrylamide gel. The proteins were visualized after blotting to nitrocellulose by probing with anti-c-Abl monoclonal antibodies 24-11 or K12 (Santa Cruz Biotechnologies). Polyclonal anti-Abi-1 antisera were raised by immunization of rabbits with 100 μ g of purified GST–Abi-1 protein in complete Freund's adjuvant, with boosts of similar amounts of protein in incomplete adjuvant.

Analysis of abi-1 mRNA levels

The tissue distribution of *abi-1* mRNA was determined by RNase protection assays. An antisense RNA probe was prepared by *in vitro* transcription reactions using T3 RNA polymerase on a linearized Bluescript pSKII plasmid containing a 362-bp fragment of *abi-1* cDNA (nucleotides 884–522), according to the specifications of the manufacturer (Stratagene). The RNA probe was purified by electrophoresis on a 5% polyacrylamide/urea gel. Whole-cell RNA was prepared from 100 mg each of liver, lung, heart, brain, thymus, spleen, kidney, muscle, testis, and bone marrow of young adult mouse, and from NIH-3T3 cells and WEHI-3 cells, using the RNazol B RNA isolation method (TEL-TEST). The amount of RNA in each sample was determined by OD_{260} and by ethidium bromide staining of agarose gels. RNA (15 μ g) from each sample was used to protect the probe from RNase A plus T1 digestion for the RPA II ribonuclease protection assay (Ambion). The digestion products were resolved on a 5% polyacrylamide/urea gel and detected by autoradiography.

Overexpression of Abl-interactor-1 in NIH-3T3 cells

The *abi-1* cDNA was inserted in-frame into the pCGN expression vector (Tanaka and Herr 1990). NIH-3T3 cells were trans-

duced by the calcium phosphate precipitation method (Chen and Okayama 1987). Plasmid pWLneo provided a neomycin selection marker (Stratagene). *abi-1* expression in G418-resistant clones was determined by immunoblot analysis of whole-cell extracts with anti-Abi-1 antibody. Antisera were prepared in rabbits by immunization with recombinant GST–Abi-1 protein.

A-MuLV focus-forming assay

P160 and P90A A-MuLV virus stocks were prepared by rescuing virus from transformed nonproducer NIH-3T3 cells with Mo-MuLV helper virus. To measure susceptibility to infection, cells were exposed to the virus stocks at 1:10 serial dilutions as described (Scher and Siegler 1975). The monolayers were then maintained in Dulbecco's modified Eagle medium supplemented with 5% bovine calf serum. The foci were scored after 2 weeks of incubation.

RT assay

The retroviruses released to the culture media were harvested and passed through 0.45- μ m filters (Nalgene). The incorporation of [^{32}P]TTP into DNA on a poly(rA)/oligo(dT) substrate was assayed by standard methods (Goff et al. 1981a).

Abl tyrosine kinase assay

p160^{v-abl} protein was collected from a cell lysate (500 μ l) prepared from P160-transformed cells by anti-Abl antibody immobilized on protein A–agarose. The immobilized p160^{v-abl} was added to GST–Abi-1 (total protein concentration 1 mg/ml; 100 μ l) in kinase buffer (50 mM Tris-HCl at pH 8.0, 50 mM NaCl, 6 mM MnCl_2 , 1 mM CTP, 1 mM PMSF, 2 μ g/ml each of pepstatin, leupeptin, and aprotinin) and preincubated for 15 min. The kinase reaction was initiated by adding [γ - ^{32}P]ATP (5000 Ci/mmol) to a final concentration of 20 nM and allowed to proceed at 24°C for 15 min. The reaction was terminated by addition of ATP and EDTA to final concentrations of 10 mM each. The labeled proteins were resolved by SDS-PAGE and detected by autoradiography.

Mapping of the abi-1 gene in the mouse genome

Two oligonucleotides (5'-GGAGGCAGTTCCAAACATTG-3') and (5'-GGACACATTAAGATTAAGC-3') from the *abi-1* 3'-untranslated region were used as DNA polymerase chain reaction primer pairs to amplify mouse genomic DNA from *M. spretus* and *M. musculus* (The Jackson Laboratory). The PCR products from each animal were digested with restriction enzymes. One *DdeI* RFLP was found. The panel of DNAs from 94 *M. spretus* and *M. musculus* interspecific backcross (BSS type) animals was then analyzed for the haplotypes based on the RFLP (Rowe et al. 1994).

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