

The amphiphysin-like protein 1 (ALP1) interacts functionally with the cABL tyrosine kinase and may play a role in cytoskeletal regulation

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ABSTRACT cABL is a protooncogene, activated in a subset of human leukemias, whose protein product is a nonreceptor tyrosine kinase of unknown function. cABL has a complex structure that includes several domains and motifs found in proteins implicated in signal transduction pathways. An approach to elucidate cABL function is to identify proteins that interact directly with cABL and that may serve as regulators or effectors of its activity. To this end, a protein–interaction screen of a phage expression library was undertaken to identify proteins that interact with specific domains of cABL. An SH3-domain-containing protein has been identified that interacts with sequences in the cABL carboxyl terminus. The cDNA encoding ALP1 (*amphiphysin-like protein 1*) was isolated from a 16-day mouse embryo. ALP1 has high homology to BIN1, a recently cloned myc-interacting protein, and also shows significant homology to amphiphysin, a neuronal protein cloned from human and chicken. The amino terminus has homology to two yeast proteins, Rvs167 and Rvs161, which are involved in cell entry into stationary phase and cytoskeletal organization. ALP1 binds cABL *in vitro* and *in vivo*. Expression of ALP1 results in morphological transformation of NIH 3T3 fibroblasts in a cABL-dependent manner. The properties of ALP1 suggest that it may be involved in possible cytoskeletal functions of the cABL kinase. Additionally, these results provide further evidence for the importance of the cABL carboxyl terminus and its binding proteins in the regulation of cABL function.

The cABL protooncogene encodes a 150-kDa nonreceptor protein tyrosine kinase that was first identified as the cellular homologue of the *v-abl* oncogene product of the Abelson murine leukemia virus (1, 2). In transfected fibroblasts, cABL has been localized to the nucleus (3), but it is also found in the cytoplasm, and some of the cytoplasmic cABL is associated with actin filaments (3, 4). In several hematopoietic cell lines, endogenous cABL is mostly cytoplasmic but does show some nuclear staining (5). Although there are data suggesting a number of possible roles for cABL in cellular signaling, its biological function remains unclear.

The transforming potential of cABL is tightly regulated *in vivo* (6, 7). Overexpression of cABL in fibroblasts leads not to cell transformation but to growth arrest (8). In contrast, a number of structurally altered forms of cABL cause transformation when expressed at similar levels in the same cell types (9–11). The transforming cABL proteins are constitutively active tyrosine kinases and are primarily localized to the cytoplasm. Recently it has been reported that the tyrosine kinase function of wild-type cABL can be activated by several

signals, including DNA damage (12) and engagement of integrin receptors (13).

The cABL protein has a complex structure and contains many domains found in proteins implicated in signal transduction pathways. These include the noncatalytic Src homology domains SH2 and SH3 and the tyrosine kinase, or SH1, domain. SH2 and SH3 domains are modular domains found in a large number of proteins and are known to be involved in protein–protein interactions, particularly in the formation of complexes involved in signaling events (14). The unique C-terminal region of cABL also contains a number of functional and structural domains, such as a nuclear localization signal (3), a DNA-binding domain (15), an actin-binding domain (4, 16), and proline-rich sequences that may bind to SH3-domain-containing proteins (17–19). The presence of an actin-binding domain and association of some of the cytoplasmic cABL with the cytoskeleton suggest that cABL may function in the transference of signals from the cell surface and cytoskeleton to the nucleus. Support for this hypothesis recently has been provided by data showing that the activity and subcellular distribution of cABL is altered following engagement of integrin receptors (13).

A number of proteins, including Nck, Crk, Grb2, and members of the Abi family, have been shown to bind, *in vitro* and/or *in vivo* to proline-rich sequences in the cABL C terminus (17–21). The importance of the C-terminal region of cABL has been demonstrated in both *Drosophila* and mice. In *Drosophila*, mutants in which C-terminal regions are deleted exhibit pupal lethality, as in the case of null mutations. Further, it has been shown that the C terminus of *Drosophila* ABL (dABL) is required for proper localization (22). Mice that lack the C-terminal one-third of the cABL last exon suffer from lymphopenia, defects in the B cell compartment, runting, and early death (23, 24). In addition, mutant forms of cABL with C-terminal deletions have been found that exhibit increased transforming activity (25).

Although a number of adapter proteins have been shown to interact with the C terminus of cABL, some of these interactions have thus far been shown only *in vitro*, and as yet no biological significance for the majority of these interactions has been clearly demonstrated. To gain insight into potential functions and mechanisms of action of the cABL protein, we sought to identify proteins that interact with specific regions of the cABL molecule. Here we report the cloning and characterization of ALP1 (*amphiphysin-like protein 1*), an SH3-containing protein that interacts functionally with sites in the cABL C terminus.

MATERIALS AND METHODS

Plasmid Constructions. The probe used for screening consisted of a portion of the cABL protein produced as a

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Abbreviations: SH, Src homology; dABL, *Drosophila* ABL; GST, glutathione S-transferase; HA, hemagglutinin.

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glutathione *S*-transferase (GST) fusion. A cABL fragment from a *Hinc*II site 4 aa into the SH2 domain to a *Pvu*II site 167 aa into the C terminus was subcloned into the vector pBI3G3xK, a derivative of the baculovirus vector pAcC12, which includes the GST cDNA sequence from pGEX-3X, a heart muscle kinase (HMK) phosphorylation site, and a stop codon 3' of the site of the insert. The resulting plasmid was used to create a baculovirus that allows synthesis of a GST-cABL fusion protein in Sf9 insect cells.

To express ALP1 as a GST fusion protein, a cDNA insert encoding the full-length protein was subcloned in frame into pGEX-3X (Pharmacia). To create an ALP1 Δ SH3 mutant, the pGEX3X-ALP1 plasmid was cut at a unique *Eco*47III site approximately 15 aa upstream of the start of the SH3 domain, and *Xba*I stop linkers were added. To express ALP1 in mammalian cells, the full-length and an SH3-deleted ALP1 were cloned into a modified pCGN expression vector (26).

Cells and Viruses. Sf9, NIH 3T3, COS, and Bosc23 cells were grown as previously described (20). Baculoviruses were prepared as described (6). Stable mass populations of NIH 3T3 cells expressing wild-type and mutant ALP1 proteins were produced by transfection. The pSR α MSVtkneo vector was used to produce helper-free retroviral stocks encoding cABL wild-type or mutant forms in Bosc23 cells (27).

Antibodies. Polyclonal antibodies against ALP1 were prepared by immunization of New Zealand White rabbits with a C-terminal fragment of ALP1. ALP1 proteins were produced as GST fusion proteins, and the GST portion was cleaved by Factor X digest. Anti-ABL polyclonal antibodies and a mouse monoclonal antibody were described previously (28, 29). Additional ABL antibodies used were the K-12 polyclonal (Santa Cruz Biotechnology) for immunoprecipitation and the 8E9 monoclonal (PharMingen) for Western blotting. Monoclonal antibody against hemagglutinin (HA) (12CA5) was purchased from Boehringer Mannheim.

Expression Library Screen. A 16-day mouse embryo library (lexlox, Novagen) was screened with a GST-cABL fusion protein. The probe was first subjected to an *in vitro* ABL autokinase reaction with cold ATP to allow autophosphorylation of ABL sites. This was followed by phosphorylation of the HMK site located between the GST protein and the cABL sequences. Approximately 400,000 recombinant phage were screened by standard *in situ* nitrocellulose filter assays. Two independent phage clones were isolated that consisted of overlapping portions of the ALP1 sequence.

Immunocytochemistry. HepG2 cells were transfected using Lipofectamine (GIBCO/BRL) and using the manufacturer's recommended protocol. Cells (1×10^5) were plated to the wells of a 6-well tissue culture dish containing glass coverslips. Two days after transfection, cells were fixed for 8–10 min in 3% paraformaldehyde in PBS and permeabilized for 2–5 min in 0.5% Triton. The cells were then incubated with either anti-HA monoclonal antibody (Boehringer Mannheim) or an affinity purified anti-ALP1 polyclonal antibody. NIH 3T3 cells expressing ALP1 in the presence of wild-type or kinase-defective cABL were fixed for approximately 15 min, and permeabilized for 5 min in 0.2% Triton. The cells were then incubated with rhodamine-phalloidin (Molecular Probes).

Cell Morphology Assay. NIH 3T3 cells coexpressing wild-type and mutant forms of cABL and/or ALP1 were examined by light microscopy. Stable hygromycin-resistant lines expressing wild-type or an SH3-deletion mutant of ALP1 were infected with ABL retroviruses as described previously. Two days after infection, cells were selected with 0.7 mg/ml G418. Expression of cABL and ALP proteins and cell morphology was examined 5–10 days after infection.

RESULTS

Expression Cloning of an ABL-Interacting Protein. To identify proteins that interact with regions of the cABL tyrosine kinase

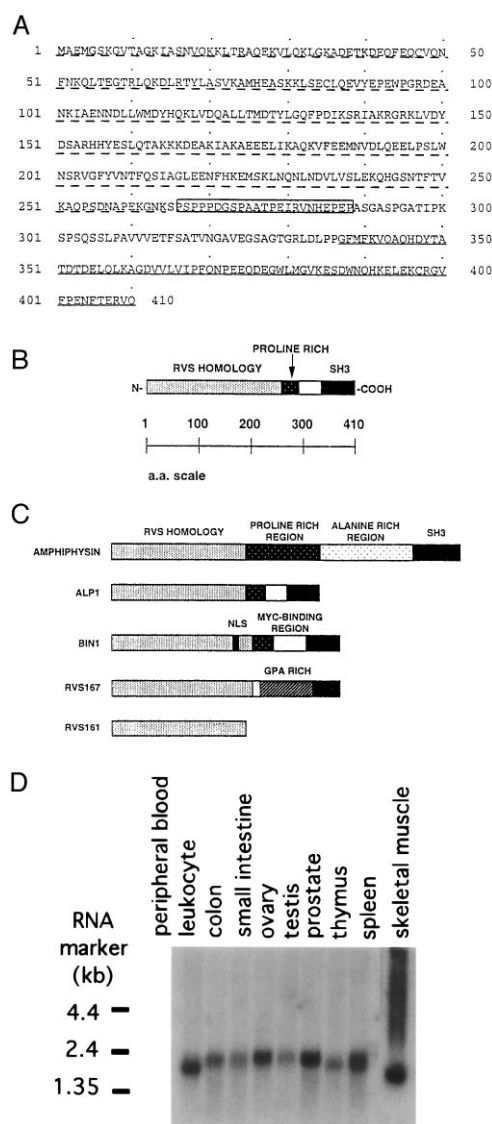


Fig. 1. Sequence, structure, and tissue distribution of ALP1. (A) Predicted amino acid sequence of murine ALP1. The SH3 domain sequence is underlined, the proline-rich region is boxed, and the RVS region is underlined with dashed lines. (B) Diagram of structural features of ALP1. (C) Structural features of ALP1-related proteins. (D) Northern blot analysis of ALP1 expression in human tissues.

implicated in signaling, a 16-day mouse embryo library was screened using a GST fusion protein probe containing the cABL SH2 and SH1 domains and an N-terminal portion of the C-terminal domain that contains several proline-rich regions. This region of the C terminus has been reported to bind to several proteins, including CRK, NCK, GRB2, Abi1, and Abi2 (17–21). Approximately 400,000 recombinant phage were screened. Two independent phage clones were isolated that encoded overlapping regions of the same sequence. The longer of the two clones was approximately 1.9 kb, and sequence analysis revealed a single ORF encoding a predicted protein of 410 aa. We have designated this protein ALP1. The amino acid sequence of ALP1 is shown in Fig. 1A.

ALP1 Encodes an SH3-Domain-Containing Protein with Homology to BIN1, Amphiphysin, and the Yeast Proteins Rvs161 and Rvs167. Analysis of the predicted amino acid sequence of ALP1 reveals several structural features (Fig. 1B). The C-terminal region of ALP1 encodes an SH3 domain (amino acids 336–410). Upstream of the SH3 domain is a proline-rich region, which contains a PXXP motif, a sequence found in known high-affinity SH3-domain-binding ligands (30,

31). A search of the database revealed that the ALP1 sequence is essentially identical to that of SH3P9, a murine protein identified in a screen for novel SH3-domain-containing proteins (32). This report, however, did not provide any data regarding function of this protein. More recently, the cloning of a novel human myc-interacting protein, BIN1, was reported (33). ALP1 is approximately 95% identical to BIN1 at the amino acid level, suggesting that ALP1/SH3P9 is the murine homologue of BIN1. Interestingly, ALP1 appears to be a splice variant of BIN1, because a 15-aa stretch containing a nuclear localization signal (NLS) and about one-third of the approximately 110-aa myc-binding region of BIN1 are absent in the ALP1 sequence (Fig. 1C). A recent report characterizing a second mammalian amphiphysin-related gene (amphiphysin II) provides further evidence for the role of alternative splicing in the production of ALP1 and related proteins (34).

ALP1 and BIN1 have the strongest overall homology with the protein amphiphysin (35), with which ALP1 is approximately 58% identical at the amino acid level (Fig. 1C). Amphiphysin was first cloned as a novel synaptic protein in chicken, and the human homologue was shown to be an autoantigen in breast-cancer-associated cases of the neurological disorder Stiff-Man syndrome (36). More recently, amphiphysin has been reported to interact with the GTPase dynamin and with the clathrin adapter AP2 (37, 38). The N-terminal region of ALP1, BIN1, and amphiphysin also has homology to two yeast proteins, RVS167 and RVS161 (39–41). Yeast with mutations in either of these genes were found to have reduced viability under starvation conditions, implicating RVS167 and RVS161 in the transition from exponential cell growth to stationary phase. Interestingly, mutations in these genes also lead to delocalization of cytoskeletal actin. In addition to the N-terminal region of homology (RVS homology domain), which is shared by all five proteins, amphiphysin, ALP1, BIN1, and RVS167 also contain a C-terminal SH3 domain (Fig. 1C).

ALP1 Is Expressed in Multiple Tissues. To investigate the expression pattern of ALP1, Northern blot analyses were performed. Poly(A)-selected RNA from several human tissues was probed with radiolabeled ALP1 cDNA. A single major transcript of approximately 2.1 kb was detected in a variety of tissues, though the message size varied slightly from tissue to tissue, with highest levels seen in skeletal muscle (Fig. 1D). The widespread nature of ALP1 expression is similar to that of the cABL tyrosine kinase.

ALP1 Localizes to Both the Nucleus and the Cytoplasm. BIN1 has been localized to the nucleus by indirect immunofluorescence of transiently transfected HepG2 cells (33). The nuclear localization of BIN1 is consistent with the presence of an NLS in the BIN1 sequence, as well as with the interaction between BIN1 and myc. The murine ALP1 sequence, though highly homologous to the sequence of the human BIN1, lacks the putative NLS. Therefore, we sought to examine the cellular localization of ALP1 in HepG2 cells. After transfection with pCGN-ALP1 (HA tagged), cells were stained with either a monoclonal antibody against the HA tag or an affinity-purified polyclonal antibody raised against the full-length ALP1 protein. Both antibodies yielded similar results. About 45% of the cells expressing the transfected ALP1 exhibited primarily nuclear staining, despite the lack of the NLS (Fig. 2 *Left*). However, ALP1 expression was not limited to the nucleus, because approximately 25% of the cells stained exhibited primarily cytoplasmic staining (Fig. 2 *Right*), whereas the remaining 30% of the cells showed staining in both compartments. The cells employed in this experiment are a heterogeneous population of exponentially growing cells. It is possible that the localization of ALP1 is regulated, for example, during distinct stages of the cell cycle.

ALP1 Binds cABL *in Vitro* and *in Vivo* but Does Not Bind c-myc. ALP1 was identified as a cABL-interacting protein via standard *in situ* nitrocellulose filter assays by using a portion of the cABL molecule as a probe. To determine whether the full-length

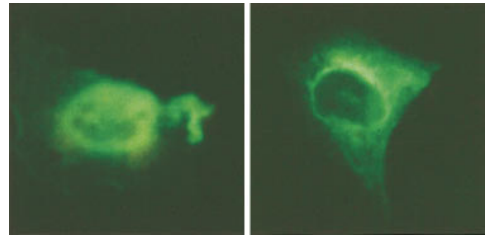


FIG. 2. Subcellular localization of ALP1. HepG2 cells were transfected with the mammalian expression plasmid pCGN-ALP1 and subjected to indirect immunofluorescence. ALP1 localizes to the nucleus (*Left*) and to the cytoplasm (*Right*).

proteins can interact in solution, a GST-ALP1 fusion protein was produced and used in an *in vitro* binding assay with full-length cABL protein produced in Sf9 insect cells. As shown in Fig. 3 *Left*, GST-ALP1 interacts with full-length cABL. Additionally, a GST-ALP1 C-terminal fusion protein, which consists primarily of the SH3 domain of ALP1, is also capable of binding to cABL (data not shown), implying that this region of ALP1 is likely to be involved in the interaction.

To examine whether cABL and ALP1 could interact *in vivo* we utilized a COS cell expression system. COS cells were transiently cotransfected with mammalian expression vectors coding for cABL and ALP1. Cell lysates were incubated with preimmune, anti-ALP, or anti-ABL sera, and the immunoprecipitates were analyzed by Western blotting using an anti-ABL monoclonal antibody. The 145-kDa cABL protein was precipitated by the anti-ALP antisera but not by the corresponding preimmune sera (Fig. 3 *Right*, lanes 1 and 2). ALP1 is also observed in anti-ABL immunoprecipitations using antibodies against regions of the cABL kinase domain and a region upstream of the kinase domain (data not shown).

BIN1 is highly homologous to ALP1. However, the ALP1 sequence lacks approximately one-third of the middle of the myc-binding domain found in BIN1. Therefore, we sought to determine whether or not ALP1 would bind c-myc. Using a mammalian expression vector that drives the expression of both human c-myc and the chimeric p210 BCR-ABL oncoprotein, the two proteins were overexpressed following transient transfection in Bosc23 cells. GST-ALP1 pulls down p210 BCR-ABL but not c-myc (data not shown).

The ALP1 SH3 Domain Interacts with Carboxyl-Terminal Sequences in cABL. The shorter of the two ALP1 clones isolated in our initial screen consisted primarily of the SH3 domain, containing approximately 50 additional aa upstream. A GST fusion with only this portion of the protein retained the

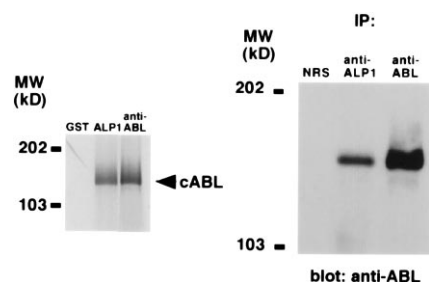


FIG. 3. ALP1 interacts with cABL. (*Left*) Full-length cABL protein was produced in Sf9 cells labeled with [³⁵S]methionine, and cell lysates were incubated with immobilized GST alone (lane 1), GST-ALP1 (lane 2), and anti-ABL antibody bound to protein A-Sepharose (PAS) beads (lane 3). Bound proteins were separated by SDS/PAGE and visualized by fluorography. (*Right*) COS cells were cotransfected with the pSR α -cABL and pCGN-ALP1 expression vectors. Lysates were immunoprecipitated with preimmune serum (lane 1), anti-ALP (lane 2), and anti-ABL (lane 3) antibodies. Immunoprecipitated proteins were analyzed by Western blotting with an anti-ABL monoclonal antibody.

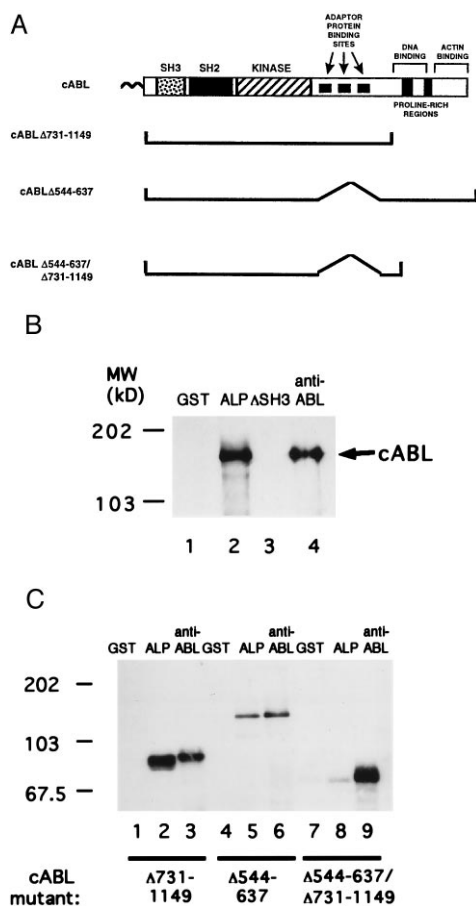


FIG. 4. The ALP1 SH3 domain interacts with C-terminal sequences in cABL. (A) Schematic of cABL and cABL mutants. (B) Full-length cABL was produced by transient transfection in COS cells. Cell lysates were incubated with GST alone (lane 1), GST-ALP1 (lane 2), GSTALP Δ SH3 (lane 3), and anti-ABL antibody bound to PAS beads. Bound proteins were separated by SDS/PAGE and analyzed by Western blotting. (C) Mutant cABL proteins were produced in COS cells. Cell lysates were incubated with GST alone (lanes 1, 4, and 7), GST-ALP1 (lanes 2, 5, and 8), or anti-ABL antibody bound to PAS beads (lanes 3, 6, and 9). Bound proteins were analyzed as in B.

ability to interact with full-length cABL in solution. There are several proline-rich sites in the first half of the cABL C terminus that have been reported to interact with SH3-domain-containing adapter proteins. These sites were included in the fusion protein used to screen the expression library. Therefore, it seemed likely that cABL and ALP1 might interact via an SH3-domain-proline interaction involving the ALP1 SH3 domain and sequences in the adapter-binding region of the cABL C terminus.

To investigate this possibility, an ALP1 mutant lacking the SH3 domain was created and produced as a GST fusion, which was used in an *in vitro* binding assay. As shown in Fig. 4B, the ALP1 protein deleted for the SH3 domain is unable to bind to cABL. However, deletion of the cABL proline-rich sequences implicated in binding to the SH3-containing adapter proteins (cABL Δ 544-637) did not abrogate binding of ALP1 to cABL (Fig. 4C, lanes 4-6), suggesting a second binding site for ALP1 in the cABL protein. There is another region of the cABL C terminus that contains several proline-rich sequences and may provide another potential site of interaction with the ALP1 SH3 domain. Additionally, ALP1 contains several proline-rich regions of its own, so it was also possible that it could interact with the cABL SH3 domain via these regions.

To determine which portion of cABL, in addition to the adapter-binding region, is involved in cABL's interaction with

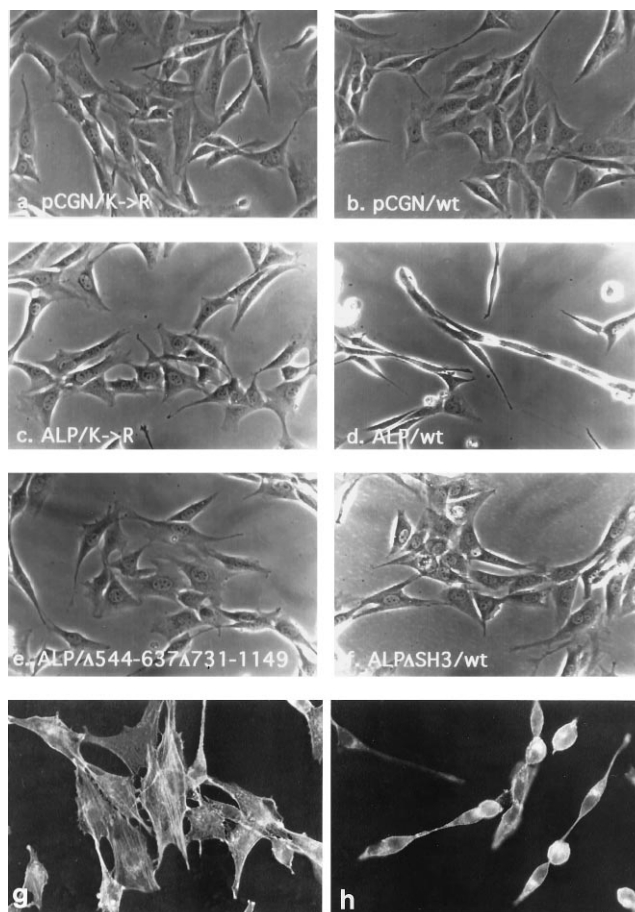


FIG. 5. Coexpression of ALP1 and cABL results in morphological transformation of NIH 3T3 cells. NIH 3T3 cells were transfected with the pCGN vector, pCGN-ALP1, or pCGN-ALP1 Δ SH3, and stable lines were selected. The cell lines were infected with retroviruses expressing kinase-inactive (a and c), wild-type (b, d, and f), and Δ 544-637/ Δ 731-1149 (e) cABL forms. Rhodamine-phalloidin staining of pCGN-ALP1 cells infected with kinase inactive or wild-type cABL is shown in g and h, respectively.

ALP1, several cABL deletion mutants were created (Fig. 4A). These mutants were expressed in COS cells and subjected to the *in vitro* binding assay with GST-ALP1. A C-terminal truncation (cABL Δ 731-1149) mutant, which lacks the downstream proline-rich regions in the cABL C terminus, retains the ability to bind ALP1 (Fig. 4C). However, when this truncation was combined with the adapter region deletion mutant, little or no binding to ALP1 remained (Fig. 4C). Deletion of the cABL SH3 domain, alone or in combination with the proline-rich adapter-binding region, had no effect on ALP1 binding (data not shown). Thus, it appears that ALP1 can bind to multiple proline-rich sites in the cABL C terminus via its SH3 domain.

Coexpression of ALP1 and cABL Results in Dramatic Changes in Cell Morphology. It has been shown that overexpression of wild-type cABL in NIH 3T3 cells results in growth

Table 1. Effect of ALP1 on BCR-ABL transformation of Rat1 cells

Cell line	No. of agar colonies (10^4 cells)
Rat1/pCGN	221
Rat1/ALP(1.3)	283
Rat1/ALP(2.5)	546

Rat1 cells were infected with retrovirus encoding p185 BCR-ABL. Mass populations of drug-selected cells were seeded at a density of 2×10^4 cells per 6-cm² dish. Colonies were counted after ~3 weeks. Colony numbers from two plates per assay were averaged.

arrest and in counterselection of the cells expressing the cABL protein. Wild-type cABL can be transiently overexpressed via retroviral infection, but after 2 weeks of selection of infected cells, cABL levels decrease so that they are comparable to the endogenous cABL protein (8). To examine possible effects of ALP1 on cABL function, we coexpressed ALP1 and cABL in NIH 3T3 cells. Cells were transfected with a mammalian expression vector encoding ALP1. Several stable mass populations overexpressing ALP1 were isolated, as were several control hygromycin-resistant lines containing empty vector. These cell lines were infected with retroviruses encoding wild-type or kinase-inactive cABL (K290R), or with a vector control virus. Infected cells were collected 2–3 days postinfection and again after 21–22 days of G418 selection. Lysates were analyzed by SDS/PAGE and Western blotting for ALP1 and cABL. At 2–3 days postinfection, expression of both wild-type and kinase-inactive cABL was relatively high. After selection, cABL wild-type protein had been counterselected in the vector control cells, as has been previously described (8), and overexpression of ALP1 did not affect the loss of cABL expression (data not shown).

Although ALP1 expression did not affect counterselection of cABL in NIH 3T3 cells, there was a striking difference between cells expressing both cABL wild type and ALP1 and those cells expressing either protein alone, ALP1 with kinase inactive cABL, or the vector controls. Cells coexpressing wild-type cABL and ALP1 had a distinctive morphological phenotype and appeared to be “transformed,” taking on a more spindly, refractile appearance (Fig. 5*d*). Expression of cABL wild type or ALP1 alone had no effect on cell morphology. Further, the morphological changes observed required an active cABL kinase, because the kinase-deficient cABL K290R mutant in combination with ALP1 failed to elicit the morphological changes (Fig. 5*a–c*). The interaction of ALP1 with ABL proteins also may have an effect on the function of oncogenic forms of ABL. In a Rat1 agar colony formation system, overexpression of ALP1 has a mildly stimulatory effect on transformation by the chimeric BCR-ABL tyrosine kinase, as measured by agar colony formation (Table 1). The increase in agar colony formation correlates with the level of ALP1 expression, which is greater in the Rat1/ALP (2.5) cell line. In this assay, Rat1 lines stably expressing ALP1 did not form colonies in the absence of BCR-ABL expression (data not shown).

Interestingly, although overexpression of ALP1 and cABL leads to a morphology resembling that of transformed cells, and a wild-type kinase domain is required for this effect, it does not appear to lead to an activation of cABL kinase activity, as measured by an *in vitro* kinase assay, and these cells do not appear to be fully transformed, in terms of ability to grow in soft agar (data not shown). Nor does ALP1 appear to be a substrate for the cABL kinase. However, ALP1 overexpression may affect cABL localization and/or the composition of protein complexes associated with cABL. It should be noted that BIN1 might also affect cABL localization and/or complex formation, because it shares an SH3 domain with ALP1 and thus is likely to bind nuclear cABL.

We further sought to determine whether the effect of ALP1 coexpression with cABL on cellular morphology was dependent on the ability of the two proteins to interact. To this end, we utilized the ALP1 Δ SH3 and the cABL Δ 544–637/ Δ 731–1149 mutants. Stable NIH 3T3 lines expressing the SH3 deletion mutant of ALP1, which fails to bind cABL, were created, as was retrovirus encoding the doubly deleted cABL Δ 544–637/ Δ 731–1149 mutant. Although coexpression of the wild-type proteins leads to dramatic effects on cellular morphology, substitution of a noninteracting mutant of either cABL or ALP1 abrogates these effects (Fig. 5*e* and *f*). Thus, cABL tyrosine kinase activity and direct interaction between cABL and ALP1 are required for morphological alteration of the cells.

Finally, to further examine the change in cell morphology induced by coexpression of ALP1 and cABL, we performed rhodamine/phalloidin staining of the NIH 3T3 cells expressing ALP1 and cABL K290R or wild-type cABL. Actin stress fibers are clearly visible in the K290R-expressing cells in the presence of ALP1. However, cells coexpressing ALP1 with wild-type cABL acquired a spindly, rounded-up morphology with few, if any, visible stress fibers (Fig. 5*g* and *h*).

DISCUSSION

Using a protein-interaction cloning technique, we have identified a cDNA encoding a new cABL-binding protein, ALP1. ALP1 interacts with cABL C-terminal sequences via its SH3 domain. Coexpression of ALP1 with cABL leads to an alteration of cell morphology. This alteration in cABL function occurs without apparent concomitant activation of cABL kinase activity, although a wild-type cABL kinase is required for the effect.

Sequence analysis revealed that ALP1 is most highly related to the recently cloned protein BIN1 (33). BIN1 was cloned in a two-hybrid screen to identify proteins that interact with highly conserved N-terminal regions of the myc protein. BIN1 localizes to the nucleus and, when expressed in rat embryo fibroblasts, decreases Ras/myc focus formation. Although ALP1 is more than 90% identical to BIN1, it appears to be a splice variant, because it is missing two regions of potential functional importance. ALP1 lacks the nuclear localization signal described for BIN1 and in addition is missing approximately one-third of the myc-binding domain. It is therefore possible that alternative splicing of the ALP1/BIN1 message could play a regulatory role by producing multiple proteins that could perform several different functions in various subcellular compartments. For example, BIN1 is reported to have properties suggestive of a tumor suppressor, including an inhibitory effect on myc transformation (33). This effect requires the myc-binding region of BIN1, part of which is absent in ALP1. Further, ALP1 does not bind to myc. Therefore, ALP1 is not predicted to inhibit transformation by myc. Consistent with this prediction, coexpression of ALP1 with p185 BCR-ABL in Rat1 cells does not result in decreased colony formation in soft agar but rather produces an increase of up to 3.5-fold in transforming efficiency. The possibility that splicing produces proteins that may perform different functions is further supported by the observation that although BIN1 is a nuclear protein, ALP1 can localize to the cytoplasm as well as to the nucleus.

Alternatively, ALP1 may function to inhibit/antagonize BIN1 activity in the nucleus. Because both ALP1 and BIN1 share a common SH3 domain, the two proteins may compete for binding to the cABL C terminus *in vivo* and may function as distinct regulators or effectors of the cABL tyrosine kinase in the cell. ALP1 and BIN1 may regulate the formation of complexes of cABL with the c-myc transcription factor. These proteins may also be involved more generally as regulators or effectors of potential cABL transcriptional activities (42–45).

ALP1 and BIN1 are most highly related to the protein amphiphysin, which was first identified in chicken as a novel protein associated with synaptic vesicles (35). The human homologue has been cloned and identified as the 128-kDa autoantigen in breast-cancer-associated cases of the neurological disease Stiff-Man syndrome (36). More recent studies have shown that amphiphysin can interact with dynamin, a GTPase implicated in the internalization of clathrin-coated vesicles, via its SH3 domain, and with the clathrin adapter AP2 via another site (37, 38). Therefore, it has been suggested that amphiphysin may play a role in synaptic vesicle endocytosis, perhaps by binding AP2 and dynamin simultaneously. Further evidence for this type of role has been provided by a recent report in which microinjection of amphiphysin's SH3 domain into the lamprey giant reticulospinal synapse was shown to inhibit

synaptic vesicle endocytosis (46). It remains to be determined whether ALP1 plays a role in endocytosis.

ALP1 has a broader tissue distribution than amphiphysin, which is expressed primarily in the brain, with lower levels in the adrenal gland, pituitary, and testis (35). ALP1 message is highly expressed in skeletal muscle. A potential role for cABL in muscle has been suggested by studies of dABL. dABL is highly expressed in the developing central nervous system, in mesoderm cells as they differentiate into the visceral and somatic musculature, and subsequently at the muscle attachment sites of the somatic muscle (47). In the absence of both dABL and disabled, a protein shown to interact genetically with dABL, embryos at late stage 16 had obvious defects in somatic musculature (47). Interestingly, dABL/disabled double mutants also exhibit defective axon connections (48), and a recent report in which the expression of alternatively spliced products of the ALP1/amphiphysin II locus was examined suggests that certain splice variants are preferentially expressed in the brain (34). These data point to potential roles for both cABL and ALP1 in brain as well as muscle.

A role for ABL proteins in the cytoskeleton has been suggested by the presence of an actin-binding domain in the C terminus (4, 16) and the localization of some of these proteins to the actin cytoskeleton (3, 4, 49). This role is further supported by a report showing activation of cABL in response to integrin activation (13) and altered adhesion in cells expressing the oncogenic BCR-ABL protein (50). ALP1 has homology to proteins implicated in cytoskeletal organization and, when coexpressed with cABL in NIH 3T3 cells, causes a morphological transformation. ALP1 localizes, at least in part, to the cytoplasmic compartment, and it has homology to the RVS161 and RVS167 yeast proteins, which appear to play a role in cytoskeletal organization. Yeast cells with mutant forms of these ALP-related RVS proteins exhibit abnormal cell morphology when starved for nutrients, random budding patterns in diploids, and abnormal actin distribution (40, 41). Furthermore, the localization of an ALP1 isoform to the cortical cytomatrix of axons and muscle is consistent with a general role in the dynamics of the peripheral cytoskeleton (34). It is possible that ALP1 may participate in linking cABL to the regulation of cytoskeletal organization in various cell types.

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- Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. (1980) *Cell* **22**, 777–785.
- Wang, J. Y. J., Ledley, F., Goff, S., Lee, R., Groner, Y. & Baltimore, D. (1984) *Cell* **36**, 349–356.
- Van Etten, R. A., Jackson, P. & Baltimore, D. (1989) *Cell* **58**, 669–678.
- Van Etten, R. A., Jackson, P. K., Baltimore, D., Sanders, M. C., Matsudaira, P. T. & Janmey, P. A. (1994) *J. Cell Biol.* **124**, 325–340.
- Wetzler, M., Talpaz, M., Van Etten, R. A., Hirsh-Ginsberg, C., Beran, M. & Kurzrock, R. (1993) *J. Clin. Invest.* **92**, 1925–1939.
- Pendergast, A. M., Muller, A. J., Havlik, M. H., Clark, R., McCormick, F. & Witte, O. N. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5927–5931.
- Mayer, B. J. & Baltimore, D. (1994) *Mol. Cell. Biol.* **14**, 2883–2894.
- Sawyers, C. L., McLaughlin, J., Goga, A., Havlik, M. & Witte, O. N. (1994) *Cell* **77**, 121–131.
- Frantz, W. M., Berger, P. & Wang, J. Y. J. (1989) *EMBO J.* **8**, 137–147.
- Jackson, P. & Baltimore, D. (1989) *EMBO J.* **8**, 449–456.
- Muller, A. J., Young, J. C., Pendergast, A. M., Pondel, M., Landau, N. R., Littman, D. R. & Witte, O. N. (1991) *Mol. Cell. Biol.* **11**, 1785–1792.
- Kharbanda, S., Ren, R., Pandey, P., Schafman, T. D., Feller, S. M., Weichselbaum, R. R. & Kufe, D. W. (1995) *Nature (London)* **376**, 785–788.
- Lewis, J. M., Baskaran, R., Taagepera, S., Schwartz, M. & Wang, J. Y. J. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 15174–15179.
- Pawson, T. (1995) *Nature (London)* **373**, 573–580.
- Kipreos, E. T. & Wang, J. Y. J. (1992) *Science* **256**, 382–385.
- McWhirter, J. R. & Wang, J. Y. J. (1993) *EMBO J.* **12**, 1533–1546.
- Ren, R., Ye, Z. S. & Baltimore, D. (1994) *Genes Dev.* **8**, 783–795.
- Feller, S. M., Knudsen, B. & Hanafusa, H. (1994) *EMBO J.* **13**, 2341–2351.
- Feller, S. M., Ren, R., Hanafusa, H. & Baltimore, D. (1994) *Trends Biochem. Sci.* **19**, 453–458.
- Dai, Z. & Pendergast, A. M. (1995) *Genes Dev.* **9**, 2569–2582.
- Shi, Y., Alin, K. & Goff, S. P. (1995) *Genes Dev.* **9**, 2583–2597.
- Henkemeyer, M., West, S. R., Gertler, F. B. & Hoffmann, F. M. (1990) *Cell* **63**, 949–960.
- Schwartzberg, P. L., Stall, A. M., Hardin, J. D., Bowdish, K. S., Humaran, T., Boast, S., Harbison, M. L., Robertson, E. J. & Goff, S. P. (1991) *Cell* **65**, 1165–1185.
- Tybulewicz, V. L., Crawford, C. E., Jackson, P. K., Bronson, R. T. & Mulligan, R. C. (1991) *Cell* **65**, 1153–1163.
- Goga, A., McLaughlin, J., Pendergast, A. M., Parmar, K., Muller, A., Rosenberg, N. & Witte, O. N. (1993) *Mol. Cell. Biol.* **13**, 4967–4975.
- Tanaka, M. & Herr, W. (1990) *Cell* **60**, 375–386.
- Pear, W. S., Nolan, G. P., Scott, M. L. & Baltimore, D. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 8392–8396.
- Pendergast, A. M., Muller, A. J., Havlick, M. H., Maru, Y. & Witte, O. N. (1991) *Cell* **66**, 161–171.
- Konopka, J. B., Watanabe, S. M. & Witte, O. N. (1984) *Cell* **37**, 1035–1042.
- Rickles, R. J., Botfield, M. C., Weng, Z., Taylor, J. A., Green, O. M., Brugge, J. S. & Zoller, M. J. (1994) *EMBO J.* **13**, 5598–5604.
- Cohen, G. B., Ren, R. & Baltimore, D. (1995) *Cell* **80**, 237–248.
- Sparks, A. B., Hoffman, N. G., McConnell, S. J., Fowlkes, D. M. & Kay, B. K. (1996) *Nat. Biotechnol.* **14**, 741–744.
- Sakamuro, D., Elliot, K. J., Wechsler-Reya, R. & Pendergast, G. C. (1996) *Nat. Genet.* **14**, 69–77.
- Butler, M. H., David, C., Ochoa, G.-C., Freyberg, Z., Daniell, L., Grabs, D., Cremona, O. & De Camilli, P. (1997) *J. Cell Biol.* **137**, 1355–1367.
- Lichte, B., Veh, R. W., Meyer, H. E. & Kilimann, M. W. (1992) *EMBO J.* **11**, 2521–2530.
- De Camilli, P., Thomas, A., Cofield, R., Folli, F., Lichte, B., Piccolo, G., Meinck, H., Austoni, M., Fassetta, G., Bottazzo, G., Bates, D., Cartledge, N., Solimena, M. & Kilimann, M. W. (1993) *J. Exp. Med.* **178**, 2219–2223.
- Wang, L., Sudhof, T. C. & Anderson, R. G. W. (1995) *J. Biol. Chem.* **270**, 10079–10083.
- David, C., McPherson, P. S., Mundigl, O. & De Camilli, P. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 331–335.
- David, C., Solimena, M. & De Camilli, P. (1994) *FEBS Lett.* **351**, 73–79.
- Crouzet, M., Urdaci, M., Dulau, L. & Aigle, M. (1991) *Yeast* **7**, 727–743.
- Bauer, F., Urdaci, M., Aigle, M. & Crouzet, M. (1993) *Mol. Cell. Biol.* **13**, 5070–5084.
- Welch, P. J. & Wang, J. Y. J. (1993) *Cell* **75**, 779–790.
- Baskaran, R., Dahmus, M. E. & Wang, J. Y. J. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 11167–11171.
- Miao, Y. & Wang, J. Y. J. (1996) *J. Biol. Chem.* **271**, 22823–22830.
- Wong, K., Hardin, J. D., Boast, S., Cooper, C. L., Merrell, K. T., Doyle, T. G., Goff, S. P. & Calame, K. L. (1995) *Oncogene* **10**, 705–711.
- Shupliakov, O., Low, P., Grabs, D., Gad, H., Chen, H., David, C., Takei, K., De Camilli, P. & Brodin, L. (1997) *Science* **276**, 259–263.
- Bennett, R. L. & Hoffmann, F. M. (1992) *Development* **1992**, 953–966.
- Hoffman, F. M. (1991) *Trends Genet.* **7**, 351–355.
- McWhirter, J. R. & Wang, J. Y. J. (1991) *Mol. Cell. Biol.* **11**, 1553–1565.
- Verfaillie, C. M., McCarthy, J. B. & McGlave, P. B. (1992) *J. Clin. Invest.* **90**, 1232–1241.