

## Nonmyristoylated Abl Proteins Transform a Factor-Dependent Hematopoietic Cell Line

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**N-terminal myristoylation can promote the association of proteins with the plasma membrane, a property that is required for oncogenic variants of Src and Abl to transform fibroblastic cell types. The P210<sup>bcr/abl</sup> protein of chronic myelogenous leukemia cells is not myristoylated and does not stably transform NIH 3T3 fibroblasts; however, it will transform lymphoid and myeloid cell types in vitro and in vivo, suggesting that myristoylation is not required for Abl variants to transform hematopoietic cells. To test this hypothesis, we introduced point mutations that disrupt myristoylation into two activated Abl proteins, v-Abl and a deletion mutant of c-Abl ( $\Delta$ XB), and examined their ability to transform an interleukin-3-dependent lymphoblastoid cell line, Ba/F3. Neither of the nonmyristoylated Abl proteins transformed NIH 3T3 fibroblasts, but like P210<sup>bcr/abl</sup>, both were capable of transforming the Ba/F3 cells to factor independence and tumorigenicity. Nonmyristoylated Abl variants did not associate with the plasma membrane in the transformed Ba/F3 cells. These results demonstrate that Abl proteins can transform hematopoietic cells in the absence of membrane association and suggest that distinct functions of Abl are required for transformation of fibroblast and hematopoietic cell types.**

Numerous proteins, including the viral and cellular forms of Src and Abl (2, 17, 38, 39), undergo myristoylation, a cotranslational modification that entails removal of the first methionine residue and attachment of a 14-carbon myristoyl fatty acid moiety to the resulting N-terminal glycine (2, 29, 45). The N-terminal amino acid sequences of myristoylated proteins share only limited homology. The only absolute requirement is a glycine at the second position of the amino acid sequence (42). Mutagenesis of the N terminus of Src defines a short stretch of some six residues that is sufficient to determine myristoylation (1, 4, 18, 31). These studies demonstrate that myristoylation is involved in targeting the Src protein to associate with the plasma membrane of cells and that this function is critical for the capacity of Src to transform fibroblast cell types (4). Membrane association plays a similar role in the transforming function of Abl protein variants. The myristoylated v-Abl protein of Abelson murine leukemia virus transforms NIH 3T3 fibroblasts, but the nonmyristoylated 210-kDa Bcr/Abl fusion protein (P210<sup>bcr/abl</sup>) of chronic myelogenous leukemia cells does not (7, 24). Interestingly, attachment of myristoylated Gag sequences to the N terminus of Bcr/Abl creates a Gag/Bcr/Abl protein that efficiently transforms NIH 3T3 fibroblasts (7). Though it fails to transform fibroblasts, P210<sup>bcr/abl</sup> readily transforms interleukin-3 (IL-3)-dependent hematopoietic cells to growth factor independence and tumorigenicity (5, 13) and induces a variety of hematopoietic neoplasms in mouse model systems (8, 9, 14, 15, 21). These observations

suggest that myristoylation may not be as critical for transformation of hematopoietic cell types as it is for fibroblasts. To determine whether myristoylation is dispensable for hematopoietic cell transformation, myristoylation-defective mutants of v-Abl and a transforming SH3 deletion mutant of c-Abl ( $\Delta$ XB [17]) were assayed for their capacity to mimic the biological activity of P210<sup>bcr/abl</sup>. Like P210<sup>bcr/abl</sup>, neither of the nonmyristoylated mutants transformed NIH 3T3 fibroblasts, but both retained the capacity to render the IL-3-dependent lymphoblastoid cell line Ba/F3 (30) growth factor independent and tumorigenic. The nonmyristoylated variants of Abl did not associate with the plasma membrane in Ba/F3 cells. These results suggest that myristoylation-dependent plasma membrane association is necessary for NIH 3T3 fibroblast transformation but not for the capacity of Abl protein variants to transform hematopoietic cells.

### MATERIALS AND METHODS

**Constructs and cell lines.** The constructs used in these experiments were derivatives of the pucLJ retroviral vector (Fig. 1). The cDNA inserts for type IV c-Abl, the N-terminal type IV c-Abl deletion mutant  $\Delta$ XB (17), v-Abl, and P210<sup>bcr/abl</sup> (5) have been described previously. A myristoylation-defective mutant of v-Abl was made by oligonucleotide-directed mutagenesis of the glycine codon at amino acid position number two of the Moloney gag p15 sequences of v-*abl*. The GGC codon for glycine was mutated to GAC, which specifies an aspartic acid residue, resulting in an N-terminal glycine-minus mutant of the v-Abl protein (Gly<sup>-</sup>v-Abl). In the same manner, a myristoylation-deficient mutant of the N-terminal type IV c-Abl deletion mutant  $\Delta$ XB (17) was constructed by oligonucleotide-directed mutagenesis. The Gly<sup>-</sup> $\Delta$ XB construct is identical to wild-type  $\Delta$ XB except for a two-base-pair mutation in the second codon of the *abl* sequence which specifies an alanine residue (GCT)

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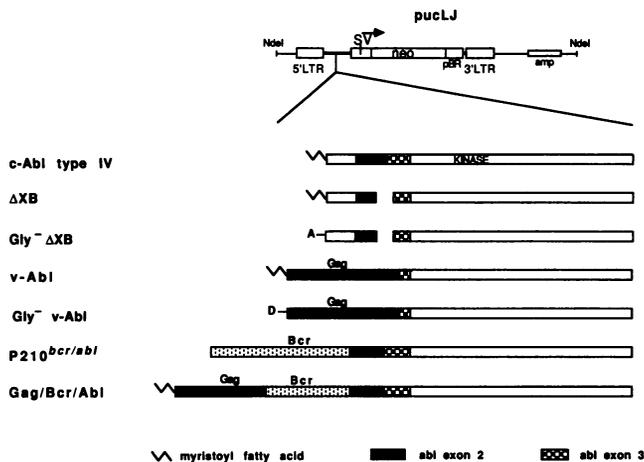


FIG. 1. Constructs for the expression of Abl proteins. The pucLJ vector is a derivative of pLJ (A. Korman and R. C. Mulligan, Whitehead Institute). The pucLJ vector expresses a cDNA insert from the 5' retroviral long terminal repeat (LTR) and a selectable marker gene (*neo*, encoding resistance to the antibiotic G418) from an internal simian virus 40 (SV) promoter. Coding sequences for several Abl protein variants were cloned into the vectors for use in expression studies in NIH 3T3 fibroblasts or IL-3-dependent Ba/F3 cells. These constructs are shown schematically to illustrate the protein structures predicted for the cloned cDNA inserts. The Gag/Bcr/Abl protein derives from a recombinant viral isolate (7) and is shown here for structural comparison. A cloned facsimile has been described elsewhere (44).

instead of glycine (GGG). Because of the absolute substrate specificity of eukaryotic *N*-myristoyltransferases for *N*-terminal glycine (41, 42), the Gly<sup>-</sup> v-Abl and Gly<sup>-</sup> ΔXB *N* termini cannot be myristoylated. The constructs were assayed for their capacity to transform NIH 3T3 fibroblasts by one of the following two methods. Either constructs were cotransfected with a plasmid specifying Moloney helper virus (pZAP) onto NIH 3T3 cells, or a helper-free retroviral producer line was generated and viral supernatant was used to infect NIH 3T3 cells. Helper-free producer lines were created by electroporating a linearized plasmid construct into  $\psi$ -2 cells (26) and selecting for stable integrants in G418 (1 mg/ml). The transfected or infected NIH 3T3 cells were grown to confluence and scored for the appearance of transformed foci after 14 days. Transformation was assessed by the capacity to form foci of rounded, refractile cells above a flat, confluent monolayer. For cells that appeared morphologically transformed, anchorage-independent growth was verified by the ability to form colonies when suspended in semisolid medium containing Bacto-Agar (0.3%). Constructs were introduced by electroporation into the IL-3-dependent lymphoblastoid cell line Ba/F3 (30). For electroporation,  $2 \times 10^6$  to  $5 \times 10^6$  cells were washed and resuspended in 1 ml of phosphate-buffered saline; 20 to 40  $\mu$ g of linear plasmid DNA in Tris-EDTA was added to the cells, and electroporation was performed in a Bio-Rad Gene Pulser (960  $\mu$ F, 250 mV). Electroporated cells were kept on ice for 5 min and then transferred directly to growth media. Cells were grown for 2 days prior to selection in G418 (2 mg/ml). G418-resistant populations were washed twice in phosphate-buffered saline and then split into media lacking a source of IL-3 in order to select for factor-independent growth. The criteria for autonomous growth in the absence of IL-3 and tumorigenicity in nude mice were previously described (5).

**Immunoprecipitation.** Cell lysates were incubated with anti-Abl antisera (pEX4 and pEX5) and processed for an *in vitro* immune complex kinase assay as previously described (22). Immunoprecipitated proteins were displayed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and visualized by autoradiography. Equivalent numbers of cells were processed for each lane.

**Immunofluorescence.** Fibroblasts were grown on glass coverslips and then fixed and permeabilized in methanol and acetone as described previously (43). Ba/F3 cells were pelleted onto coverslips by cyto centrifugation (Shandon Instruments) and fixed in ethanol-acetic acid (3:1) for 15 min at room temperature and then in 95% ethanol for 5 min at room temperature, and then were air dried. The primary antibody was a mixture of crude anti-Abl sera (pEX4 and pEX5). The secondary antibody was a rhodamine-conjugated donkey anti-rabbit immunoglobulin G.

## RESULTS

**Nonmyristoylated Abl variants do not induce transformed foci on NIH 3T3 fibroblasts.** The myristoylated v-Abl protein readily transforms NIH 3T3 fibroblasts (37), but expression of the normal type IV c-Abl protein from a similar retroviral context does not (10, 17). An internal deletion of 53 amino acids near the *N* terminus of type IV c-Abl (called ΔXB) is sufficient to generate a transforming Abl protein (17). The ΔXB deletion maintains the *N*-terminal glycine of type IV c-Abl and encodes a myristoylated protein. To determine whether *N*-terminal myristoylation is required for Abl proteins to transform NIH 3T3 fibroblasts, glycine-minus mutants of the v-Abl and ΔXB proteins were introduced into NIH 3T3 fibroblasts. Virus from helper-free retroviral producer lines made with wild type v-*abl* caused the morphologic transformation of NIH 3T3 cells ( $>10^2$  foci per ml), but viral stocks made with the mutant Gly<sup>-</sup> v-*abl* construct failed to induce focus formation upon infection of NIH 3T3 fibroblasts ( $<1$  focus per ml; Table 1). Both the wild-type and Gly<sup>-</sup> v-*abl* mutant viruses passed the marker for resistance to the antibiotic G418 (approximately  $4 \times 10^4$  G418 CFU/ml) and induced expression of a 160-kDa phosphoprotein in infected cells (data not shown). Expression of the myristoylation-defective Gly<sup>-</sup> ΔXB protein in NIH 3T3 cells likewise did not yield transformed foci ( $<1$  focus per  $\mu$ g of plasmid), in contrast with the myristoylated ΔXB construct, which efficiently transformed NIH 3T3 fibroblasts ( $>10^2$  foci per  $\mu$ g of plasmid). We experienced difficulty in isolating nontransformed cell lines that expressed high levels of either of the Gly<sup>-</sup> Abl proteins. However, as shown below, the constructs specifying Gly<sup>-</sup> v-Abl and Gly<sup>-</sup> ΔXB express the proteins efficiently in Ba/F3 cells.

**Transformation of IL-3-dependent Ba/F3 cells by myristoylated Abl protein variants.** Constructs specifying various Abl proteins were introduced into Ba/F3 cells by electroporation (to avoid complications of retroviral recombination). Cell lines resistant to the antibiotic G418 were isolated and tested for their capacity to grow in the absence of growth factor. Introduction into Ba/F3 cells of an empty vector specifying resistance to G418 or one specifying the normal type IV c-Abl protein yielded G418-resistant cell lines that retained their dependence on exogenous IL-3 for growth. Previously, it had been demonstrated that the v-Abl protein could render Ba/F3 cells factor independent (27). In a similar manner, Ba/F3 cells that express the ΔXB protein could be selected to grow in the absence of IL-3. This factor-independent cell line expressing ΔXB was tumorigenic in nude mice (Table 1).

TABLE 1. Properties of NIH 3T3 fibroblasts and Ba/F3 cells expressing Abl protein variants

Protein	Transforms NIH 3T3 cells <sup>a</sup>	Renders Ba/F3 cells factor independent <sup>b</sup>	Tumorigenic in nude mice <sup>c</sup> (no. of mice with tumors/no. challenged)
c-Abl type IV	No	No	No (0/5)
$\Delta$ XB	Yes	Yes	Yes (3/3)
Gly <sup>-</sup> $\Delta$ XB	No	Yes	Yes (2/2)
v-Abl	Yes	Yes	Yes (9/9)
Gly <sup>-</sup> v-Abl	No	Yes	Yes (3/3)
P210 <sup>bcr/abl</sup>	No	Yes	Yes (12/12)

<sup>a</sup> The properties of type IV c-Abl,  $\Delta$ XB, and P210<sup>bcr/abl</sup> in fibroblasts (7, 17) and of wild-type v-Abl and P210<sup>bcr/abl</sup> in Ba/F3 cells (5) were described previously. For the wild-type v-*abl*, Gly<sup>-</sup> v-*abl*, and P210<sup>bcr/abl</sup> constructs, focus-forming titers were compared in a helper virus-free system. In the presence of helper virus, the Gly<sup>-</sup> v-*abl* construct underwent recombination to yield small numbers of transformed foci that carried novel Gag/Abl fusion proteins of altered size (data not shown), reminiscent of the generation of the Gag/Bcr/Abl transforming variant (7).

<sup>b</sup> Electroporated Ba/F3 cells were first selected in G418 at 2 mg/ml and then split into media lacking a source of IL-3 in order to select for factor-independent cell growth. Cell lines from which no factor-independent clones arose were weaned off IL-3 gradually in an attempt to select for clones with intermediate requirements for IL-3. Only cell lines which proliferated in the complete absence of exogenous IL-3 had a stable phenotype. By examining conditioned medium from the factor-independent cell lines, it was determined that none produced a growth factor that could support the proliferation of the parental Ba/F3 cell line.

<sup>c</sup> Factor-dependent and -independent Ba/F3 cell lines were injected subcutaneously into nude mice as described previously (5). Animals were observed for the development of large nonregressing malignant tumors. Cell lines were considered nontumorigenic if the injected mice remained tumor-free for several months.

**Transformation of Ba/F3 cells by nonmyristoylated Abl proteins.** To determine whether myristoylation is required for the ability of v-Abl and  $\Delta$ XB to transform Ba/F3 cells, constructs specifying Gly<sup>-</sup> variants of these proteins were introduced into Ba/F3 cells. G418-resistant cell populations were selected in media lacking a source of IL-3, and factor-independent cell lines were obtained, each of which formed tumors when injected into nude mice (Table 1).

**Abl expression in Ba/F3 cell lines.** Ba/F3 cell lines were analyzed to determine whether Abl proteins of the predicted size were being expressed. Cell extracts were immunoprecipitated with antisera directed against C-terminal Abl determinants and processed for in vitro immune complex kinase assay (22). Denaturing polyacrylamide gel electrophoresis followed by autoradiography allowed for the detection of the expected protein in each of the tested samples (Fig. 2). To verify that the Gly<sup>-</sup> v-Abl protein expressed in factor-independent Ba/F3 cell lines indeed lacked myristoylation, Ba/F3 cell lines expressing Gly<sup>-</sup> v-Abl, P210<sup>bcr/abl</sup>, and wild-type v-Abl were metabolically labelled with either [<sup>35</sup>S]methionine or [<sup>3</sup>H]myristate, immunoprecipitated with anti-Abl antisera, separated by denaturing polyacrylamide gel electrophoresis, and analyzed by autoradiography. Cells labelled with [<sup>35</sup>S]methionine expressed the relevant Abl protein forms (Fig. 3A), but only the wild-type v-Abl protein incorporated the [<sup>3</sup>H]myristate label (Fig. 3B). Incorporation of [<sup>3</sup>H]myristate into the transforming protein was not detected in the cells expressing the Gly<sup>-</sup> v-Abl or P210<sup>bcr/abl</sup> protein, although twice as many cells expressing Gly<sup>-</sup> v-Abl were labelled as for the wild-type v-Abl. Moreover, the structure of the Gly<sup>-</sup> v-*abl* construct introduced into the Ba/F3 cells was analyzed by Southern hybridization and shown to be unrearranged (data not shown). These results

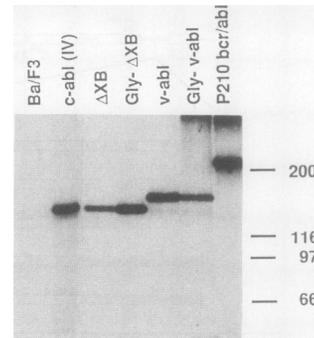


FIG. 2. Immunoprecipitation analysis of Ba/F3 cell lines expressing Abl protein variants. Lanes (from left to right) contained samples as follows. Uninfected Ba/F3 cells and a Ba/F3 cell line expressing the type IV c-Abl protein were maintained in medium supplemented with WEHI-3B conditioned medium as a source of IL-3; Ba/F3 cell lines expressing the  $\Delta$ XB, Gly<sup>-</sup>  $\Delta$ XB, v-Abl, Gly<sup>-</sup> v-Abl, and P210<sup>bcr/abl</sup> proteins were all factor independent and could be maintained in media without added IL-3. The first two lanes were taken from an independent experiment run under conditions comparable to those used for the other lanes. Sizes are shown in kilodaltons.

indicate that the Gly<sup>-</sup> v-Abl protein expressed in Ba/F3 cells lacks the myristoyl moiety. Myristoylation does not appear to be required for Abl proteins to transform the hematopoietic cell line Ba/F3.

**Immunofluorescent localization of myristoylated and nonmyristoylated Abl proteins.** The subcellular localization of various myristoylated and nonmyristoylated Abl protein forms was assessed by indirect immunofluorescence. When

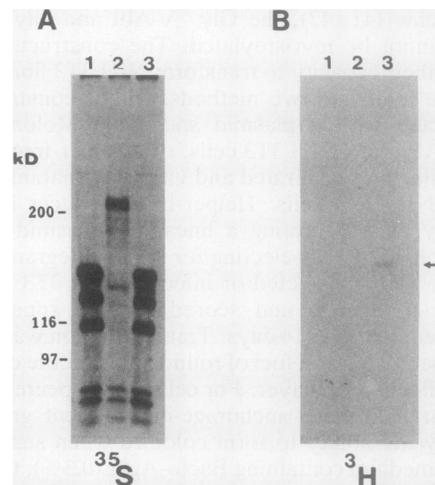


FIG. 3. Immunoprecipitation analysis of metabolically labelled, factor-independent Ba/F3 cell lines. Cell lines expressing the Gly<sup>-</sup> v-Abl (lane 1), P210<sup>bcr/abl</sup> (lane 2), and wild-type v-Abl (lane 3) proteins were labelled with either [<sup>35</sup>S]methionine for 4 h (A) or [<sup>3</sup>H]myristate for 25 min (B) and processed for immunoprecipitation analysis with anti-Abl antisera (pEX4 and pEX5). Twice as many cells were processed for the Gly<sup>-</sup> v-Abl mutant than for samples in the other lanes. Proteins were displayed by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography. Panel A was exposed for 24 h. The prominent proteins of 116 and 140 kDa in lanes 1 and 3 represent proteolytic breakdown products of the 160-kDa v-Abl protein frequently observed in these preparations. The gel in panel B was soaked in Autofluor, dried, and exposed for 72 days. The arrow denotes the position of the 160-kDa [<sup>3</sup>H]myristate-labelled v-Abl protein.

expressed in NIH 3T3 fibroblasts, the nontransforming, nonmyristoylated P210<sup>bcr/abl</sup> and Gly<sup>-</sup> v-Abl proteins localized to the cytoplasm and were not detectably associated with the plasma membrane (Fig. 4C and G). An association of the P210<sup>bcr/abl</sup> protein with actin filaments was apparent, as described previously (28, 43). The transforming, myristoylated Gag/Bcr/Abl and v-Abl variants, on the other hand, associated in part with the plasma membrane (Fig. 4A and E). These results argue that the presence of the myristoyl moiety targeted a portion of the Abl protein to associate with the plasma membrane in NIH 3T3 fibroblasts. To examine the possibility that the various Abl proteins might localize differently in hematopoietic cells, their subcellular distribution was determined in Ba/F3 cells. The myristoylated proteins v-Abl and ΔXB were mainly cytoplasmic but associated in part with the plasma membrane (Fig. 5A and C). By contrast, the nonmyristoylated Gly<sup>-</sup> v-Abl, Gly<sup>-</sup> ΔXB, and P210<sup>bcr/abl</sup> proteins showed only a cytoplasmic distribution and lacked the prominent plasma membrane staining (Fig. 5B, D, and F).

## DISCUSSION

**Myristoylation and transformation.** The nonmyristoylated P210<sup>bcr/abl</sup> protein associated with human chronic myelogenous leukemia readily transforms hematopoietic cell types in culture and in animal model systems (6), suggesting that myristoylation is not critical for the transforming activity of Abl proteins in hematopoietic cells. To verify this hypothesis, we created two nonmyristoylated point mutants of transforming Abl proteins, Gly<sup>-</sup> v-Abl and Gly<sup>-</sup> ΔXB, and showed that these forms behaved similarly to P210<sup>bcr/abl</sup>, i.e., they failed to transform NIH 3T3 fibroblasts but retained the capacity to transform the IL-3-dependent hematopoietic cell line Ba/F3. The subcellular localization of the various myristoylated and nonmyristoylated Abl variants argues that myristoylation targets some portion of the Abl protein to associate intimately with the cell's plasma membrane. The results presented above and in a previous study (7) suggest that myristoylation is required for Abl protein variants to transform NIH 3T3 fibroblasts. However, myristoylation-dependent membrane association appears irrelevant for transformation of hematopoietic cells.

Our attempts to generate stable nontransformed NIH 3T3 fibroblast cell lines that express high levels of the nonmyristoylated Gly<sup>-</sup> Abl variants proved unsuccessful, owing to the known toxicity of Abl proteins (46). Thus, we cannot rigorously exclude the possibility that myristoylation-deficient forms of the Abl protein might transform NIH 3T3 fibroblasts if high enough levels of protein could be stably achieved. However, we believe this to be unlikely, because even high levels of the P210<sup>bcr/abl</sup> protein fail to transform NIH 3T3 cells (7, 23). We are more compelled by the likelihood that myristoylation-dependent membrane association is critical for transformation of NIH 3T3 fibroblasts because of the particular transformation requirements of this adherent cell type. Alternatively, nonmyristoylated variants may be inherently unstable in NIH 3T3 fibroblasts or more stable in Ba/F3 cells. P210<sup>bcr/abl</sup> is weakly transforming for Rat-1 fibroblasts (23). It would be interesting to determine whether Bcr/Abl or other nonmyristoylated Abl variants associate with the plasma membrane or are more stable in Rat-1 cells, or whether distinct substrates are phosphorylated.

**Role of membrane-associated and cytosolic forms of Abl in transformation.** Phosphorylation of membrane-associated

components that mediate contact inhibition may be critical for the transformation of anchorage-dependent cell types (16) and thus may be facilitated by targeting of transforming protein kinases to the plasma membrane by myristoylation. Integral membrane proteins that are phosphorylated only by myristoylated Src variants have been identified by using antisera directed against phosphotyrosine (12, 36). Other candidate protein substrates include elements of signal transduction pathways. Activated Abl proteins like P210<sup>bcr/abl</sup>, Gag/Bcr/Abl, v-Abl, and ΔXB associate with a phosphatidylinositol 3'-kinase in fibroblasts, but only the membrane-associated transforming variants lead to increased steady-state levels of the 3'-phosphorylated lipid phosphatidylinositol trisphosphate (44), perhaps because of accessibility to phospholipid substrates. Phosphatidylinositol trisphosphate levels correlate with mitogenesis in fibroblasts. Activation of lipid kinase pathways may be one means by which transforming Abl proteins undermine normal growth control.

Factor-dependent hematopoietic cell types such as Ba/F3 grow in suspension culture and are not subject to the constraints of contact inhibition. Instead, regulation of cell proliferation in circulating hematopoietic cells occurs in response to diffusible growth factors. By phosphorylating some component of the IL-3 signal transduction pathway in Ba/F3 cells, the Abl kinase may override the requirement for exogenous growth factor and constitutively signal cell proliferation. The critical substrate for transformation in this case may be cytosolic and accessible to nonmyristoylated forms of Abl. Interestingly, stimulation of cell proliferation can be separated from transformation for nonmyristoylated forms of Src. Nonmyristoylated Src proteins have mitogenic activity for cells in culture, suggesting that the targets for induction of cell proliferation are accessible to nonmyristoylated Src protein (3, 19). Simply activating the Abl kinase appears sufficient to transform Ba/F3 cells in the absence of myristoylation-dependent membrane association. However, it appears that two functions are necessary for transformation of NIH 3T3 fibroblasts: a kinase activity that activates an intracellular mitogenic pathway, and membrane association which enables the kinase to phosphorylate as yet undefined membrane substrates in order to undermine contact inhibition. Myristoylation provides one means of targeting Abl proteins to associate with the plasma membrane. Fusion of the v-Abl kinase domain with the transmembrane domain of the epidermal growth factor receptor provides another such means and results in a protein that readily transforms fibroblasts in culture (32).

**Role of the myristoyl moiety in protein targeting.** It is unlikely that the hydrophobicity of the acyl moiety alone mediates the association of myristoylated proteins with membranes. A significant fraction of myristoylated proteins can be found soluble in the cell (2, 25, 29). Moreover, the association of the myristoylated alanine-rich C-kinase substrate with the plasma membrane is reversibly regulated by phosphorylation, and its distribution is concentrated in adhesion plaques (40), arguing that the protein associates with a specific receptor rather than through nonspecific insertion of the lipid moiety in the membrane bilayer. Myristoylation alone is not sufficient to specify the subcellular locale of Src. Interestingly, Src associates chiefly with the plasma membrane in fibroblasts but with other cellular membranes in distinct cell types (e.g., growth cones in neurons). Certain domains of the Src N terminus complement the myristoylation function in directing the protein to particular subcellular locations (20). Myristoylation may serve as a ligand for

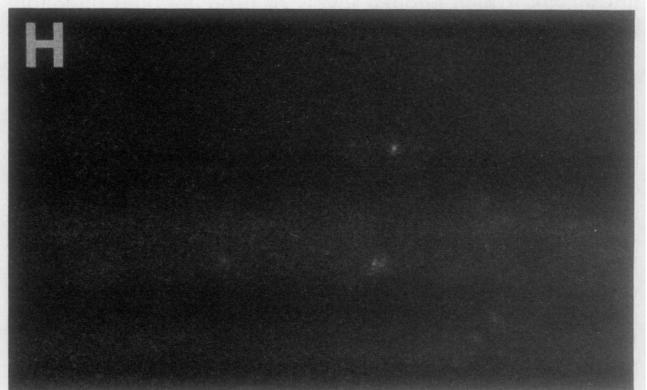
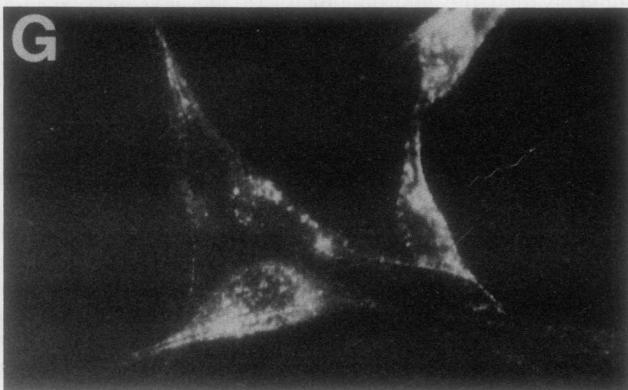
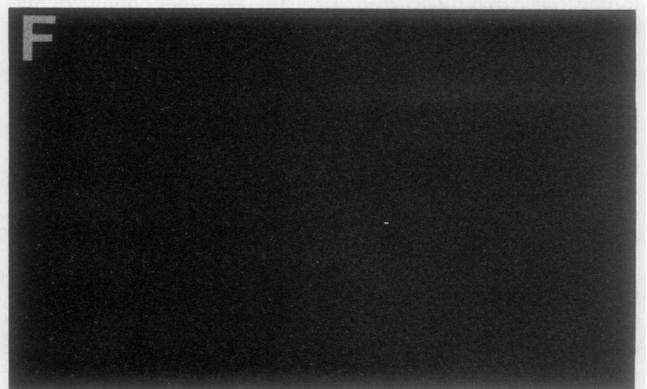
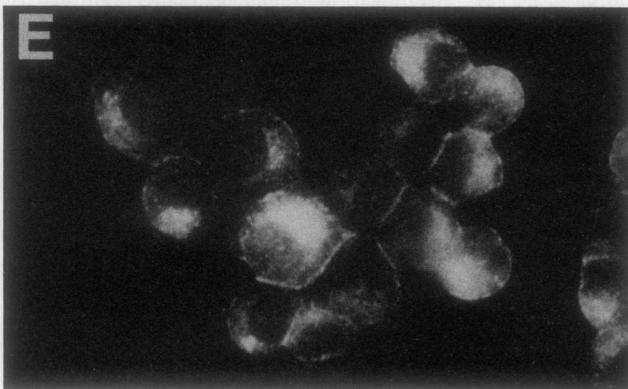
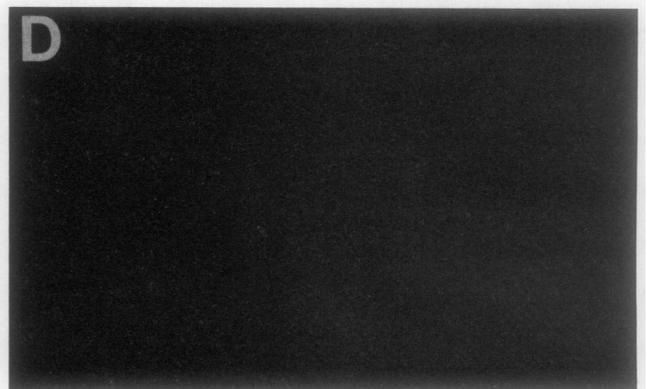
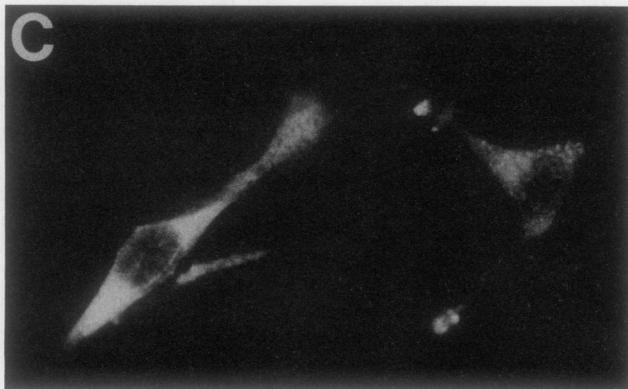
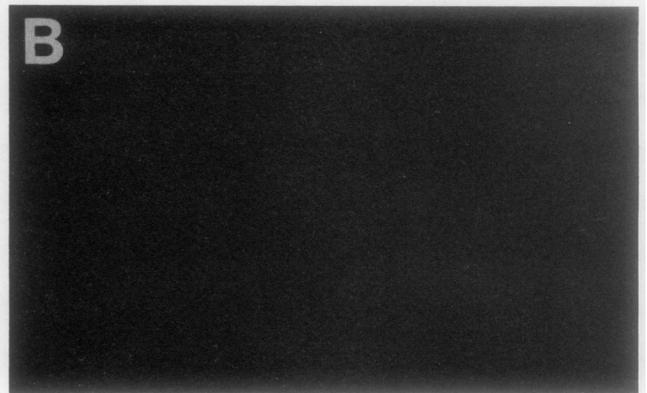


FIG. 4. Indirect immunofluorescence of NIH 3T3 fibroblasts expressing Abl protein variants. (A) Transformed cells expressing the myristoylated  $v$ -Abl protein; (B) nonimmune primary antiserum control for panel A. (C) nontransformed cells expressing the nonmyristoylated Gly<sup>-</sup>  $v$ -Abl protein (analysis was performed on an unstable clone that was undergoing loss of expression of the Abl protein); (D) nonimmune primary antiserum control for panel C; (E) transformed cells expressing the myristoylated P220 Gag/Bcr/Abl protein (7); (F) nonimmune primary antiserum control for panel E; (G) nontransformed cells expressing the nonmyristoylated P210<sup>bcrlabl</sup> protein; (H) nonimmune primary antiserum control for panel G. Note the accentuated membrane staining at areas of cell-cell contact in panels A and E.

membrane-associated receptors or docking proteins that determine specific membrane destinations within the cell (11, 33). In support of this notion, Resh (33, 34) and Resh and Ling (35) have identified a plasma membrane myristoyl

receptor which binds the Src protein. Myristoylation apparently targets only a portion of the type IV c-Abl protein in the cell to associate with the plasma membrane. The stoichiometry of myristoylation of Abl protein has not been

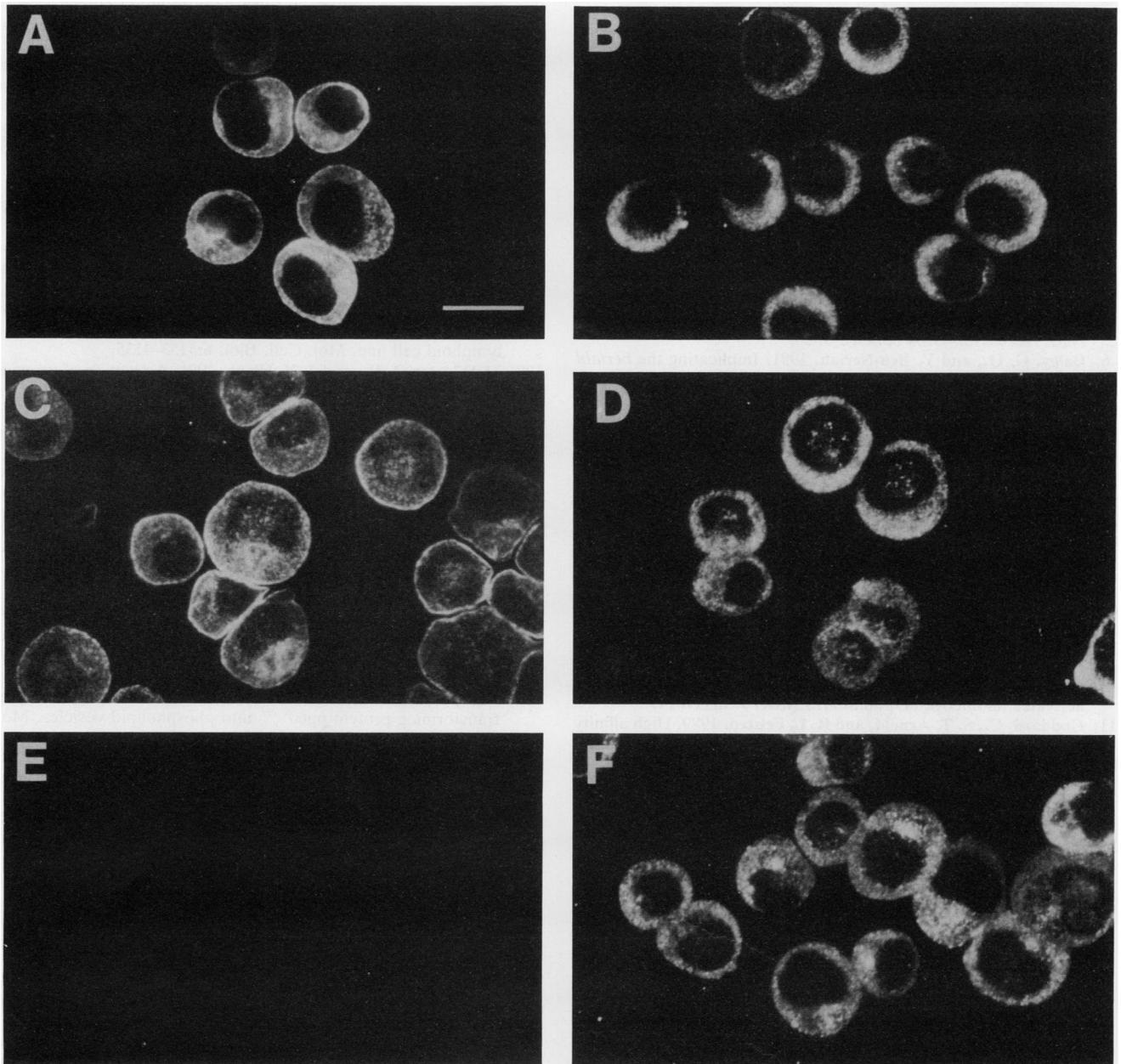


FIG. 5. Indirect immunofluorescence of Ba/F3 cells expressing Abl protein variants. (A) Myristoylated wild-type  $v$ -Abl protein; (B) nonmyristoylated Gly<sup>-</sup>  $v$ -Abl protein; (C) myristoylated  $\Delta$ XB protein; (D) nonmyristoylated Gly<sup>-</sup>  $\Delta$ XB protein; (E) parental Ba/F3 cells (control); (F) nonmyristoylated P210<sup>bcrlabl</sup> protein. Note prominent membrane staining in panels A and C. Membrane staining is lacking in panels B, D, and F.

determined, but it is highly plausible that myristoyl-Abl receptors serve to partition Abl proteins in various compartments within the cell. Myristoylation appears to be a necessary but not sufficient signal for membrane localization, a switch regulated in ways not yet understood.

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