The Extreme Carboxyl Terminus of v-Abl Is Required for Lymphoid Cell Transformation by Abelson Virus

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The v-Abl protein tyrosine kinase encoded by Abelson murine leukemia virus (Ab-MLV) induces transformation of pre-B cells in vivo and in vitro and can transform immortalized fibroblast cell lines in vitro. Although the kinase activity of the protein is required for these events, most previously studied mutants encoding truncated v-Abl proteins that lack the extreme carboxyl terminus retain high transforming capacity in NIH 3T3 cells but transform lymphocytes poorly. To understand the mechanisms responsible for poor lymphoid transformation, mutants expressing a v-Abl protein lacking portions of the COOH terminus were compared for their ability to transform pre-B cells. Although all mutants lacking sequences within the COOH terminus were compromised for lymphoid transformation, loss of amino acids in the central region of the COOH terminus, including those implicated in JAK interaction and DNA binding, decreased transformation twofold or less. In contrast, loss of the extreme COOH terminus rendered the protein unstable and led to rapid proteasome-mediated degradation, a feature that was more prominent when the protein was expressed in Ab-MLV-transformed lymphoid cells. These data indicate that the central portion of the COOH terminus is not essential for lymphoid transformation and reveal that one important function of the COOH terminus is to stabilize the v-Abl protein in lymphoid cells.

Abelson murine leukemia virus (Ab-MLV) is a replication-defective retrovirus that transforms pre-B cells and NIH 3T3 cells in vitro and induces a pre-B cell lymphoma in vivo (reviewed in reference 40). The virus encodes a single product, the v-Abl nonreceptor protein tyrosine kinase, which contains amino-terminal sequences derived from the Moloney leukemia virus gag gene fused to sequences from the c-abl protooncogene. The Gag-derived sequences localize the protein to the inner surface of the plasma membrane; abl-derived sequences specify the catalytic domain, the SH2 domain, a region which facilitates protein-protein interaction through phosphotyrosine-mediated interactions, and a long, 374-amino-acid carboxyl-terminal region. Similar to other protein tyrosine kinase oncoproteins, enzymatic activity, proper localization, and the SH2 domain are required for transformation of all cell types. In contrast, the carboxyl-terminal region is not required for transformation of NIH 3T3 cells. However, this region is necessary for efficient transformation of lymphoid cells (20, 21, 29, 39).

The mechanism by which the carboxyl terminus influences Abl function is not known. The region is unique to members of the Ab1 family and to the closely related Arg protein, but it is the least conserved portion of the proteins (18, 23, 34, 45). Despite this fact, the region contains multiple sites that may be important for function. These include proline-rich motifs that interact with the adaptor proteins Crk, Grb2, Nck, and the Abl proteins (6, 14, 35, 44, 46) and sites that bind JAK proteins (7, 8) and F- and G-actin (26, 27, 48). In addition, several serines that can be phosphorylated by protein kinase C (PKC) (31) and Cdk2 (22) are found in the COOH terminus along with a nuclear localization signal (47), a DNA binding region (22), and an RNA polymerase II binding site (2). However, because very little v-Abl protein is found in the nucleus, some of these motifs may not play important roles in transformation. Other studies (30) have suggested that signals from the COOH terminus to the Ras protein, a critical intermediate in v-Abl-mediated transformation (42), are important for lymphoid transformation.

Despite the importance of the COOH terminus for the biological effects mediated by v-Abl, mutational analyses directed toward the v-Abl COOH terminus have failed to uncover critical motifs within the region (21). The nuclear localization site and at least some of the proline-rich regions are dispensable because they are missing in the naturally selected Ab-MLV–P120 strain, a strain that retains wild-type transforming ability (24, 34, 41). However, v-Abl proteins lacking portions of the COOH terminus are highly compromised for pre-B-cell transformation in vitro (21, 29, 39), and deletion of the region that interacts with JAK proteins abolishes the ability of v-Abl to confer cytokine-independent growth to several hematopoietic cell lines (7).

To understand the way in which COOH-terminal sequences influence lymphoid cell transformation, we have analyzed a series of mutations encoding v-Abl proteins that are missing portions of the region. Our analyses reveal that removing residues that contain the major JAK binding region of the protein has only a small effect on the ability of the virus to transform pre-B cells. In contrast, a v-Abl protein lacking only the last 58 amino acids, a region which includes one of the most highly conserved regions of the COOH terminus (46), fails to transform these cells.
MATERIALS AND METHODS

Cells and viruses. NIH 3T3 cells, Ab-MLV-transformed NIH 3T3 cells, and 293T cells (11) were grown in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% fetal calf serum (Sigma) and 2 mM l-glutamine (Gibco). Ab-MLV-transformed pre-B cells were grown in RPMI 1640 medium (Gibco) with 10% fetal calf serum, 2 mM l-glutamine, and 50 μM 2-mercaptoethanol (Sigma). Viral stocks were prepared by using Ab-MLV strains in the pSRoMSV6kneo (28) or the pMIG vector (16, 49) retroviral packaging plasmid (28) as described elsewhere (25, 51). Virus was harvested 36 to 72 h posttransfection, pooled on ice, filtered through a 0.45-μm pore-size filter, and stored at −70°C. To assess the amounts of infectious Ab-MLV in pSRo-based viral stocks, 105 NIH 3T3 cells were plated on 60-mm-diameter petri dishes and were infected 24 h later with virus in the presence of 8 μg of polybrene (Sigma)/ml. After 48 h the cells were lysed, and the amount of v-AbL protein expressed by the cells was assessed by Western blotting for pSRo-based viruses (51). Viruses prepared by using the pMIG vector were titrated by infecting NIH 3T3 cells in a similar fashion and analyzing the frequency of green fluorescent protein-positive cells by flow cytometry 24 to 30 h postinfection. Bone marrow transformation assays were done as described previously by using cells from 4- to 6-week-old C57BL/6 mice. For some experiments, 2 to 106 nucleated bone marrow cells were infected and plated in agar medium; the cultures were fed 5 days later, and macroscopic colonies of primary transformants were scored at 10 days postinfection. In other experiments the infected cells were plated directly onto 35-mm-diameter dishes. The cultures were fed every 5 days and were monitored for the outgrowth of transformed cells by visual inspection. When the culture became confluent with rapidly growing pre-B cells that could be readily subcultured, the dishes were scored as transformed. In some cases the culture fluid was harvested from transformed pre-B cells, filtered through a 0.45-μm pore-size filter, and used to infect NIH 3T3 cells, which were then monitored for the appearance of typical Ab-MLV-transformed cells.

Construction and characterization of viral strains. PCR amplification was used to introduce various mutations into Ab-MLV coding sequences; the Ab-MLV genome from pUC120 (13), pSRoMSV6kneo–Ab-MLV–P120 (pSRo–P120), or pSRo–P120NotI contains an Ab-MLV genome with a NotI site immediately after base pair 2408 of the Ab-MLV–P120 genome; the v-AbL protein expressed by P120NotI virus contains four novel amino acids, Gly, Gly, Arg, and Gln, between amino acids 620 and 621 of P120 genome; the v-Abl protein expressed by P120NotI virus contains four novel amino acids of the v-Abl protein. In some instances DNA was prepared from BALB/cJ mice (38). For some experiments, 2 to 106 nucleated bone marrow cells were infected and plated in agar medium; the cultures were fed 5 days later, and macroscopic colonies of primary transformants were scored at 10 days postinfection. In other experiments the infected cells were plated directly onto 35-mm-diameter dishes. The cultures were fed every 5 days and were monitored for the outgrowth of transformed cells by visual inspection. When the culture became confluent with rapidly growing pre-B cells that could be readily subcultured, the dishes were scored as transformed. In some cases the culture fluid was harvested from transformed pre-B cells, filtered through a 0.45-μm pore-size filter, and used to infect NIH 3T3 cells, which were then monitored for the appearance of typical Ab-MLV-transformed cells.

RESULTS

The extreme 3′ end of the carboxyl terminus is required for efficient lymphoid transformation. To define the region(s) of the v-AbL carboxyl terminus required for efficient lymphoid transformation, a panel of carboxyl-terminal truncation and deletion mutants were constructed (Fig. 1A) and virus stocks were prepared. To confirm that all of the viruses expressed v-AbL proteins of the expected size, NIH 3T3 cells were infected and lysates were prepared 48 h later. Western analysis revealed that all samples contained the expected proteins (Fig. 1B) and that all of the cells, except those infected with the kinase-inactive D484N mutant, displayed elevated levels of total cellular phosphotyrosine (Fig. 1C). As expected (20, 21, 29, 39), NIH 3T3 cells infected with the COOH-terminal region mutants displayed the typical morphology characteristic of Ab-MLV-transformed cells. To test the ability of the viruses to transform pre-B cells, bone marrow cells were infected with matched titer stocks and were plated in liquid and agar media. Similar to other COOH-terminal trans-
cation mutants (21, 39), both the δ821-981 and the δ621-670/818-979 (ΔΔ) mutants that encode v-Abl proteins lacking the carboxyl-terminal third of the v-Abl protein were compromised in their ability to transform lymphoid cells (Table 1). The frequency of transformed colonies observed in the agar assay was reduced four- to fivefold, and transformation was delayed

TABLE 1. The central portion of the COOH terminus is not required for efficient pre-B-cell transformation by Ab-MLV$^a$

<table>
<thead>
<tr>
<th>Virus</th>
<th>Agar assay$^b$</th>
<th>Liquid assay$^c$</th>
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<td>Expt 2</td>
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<td>0.1 4/4 20</td>
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<td>0.1 4/4 21</td>
</tr>
<tr>
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<td>0.04 6/4 15</td>
</tr>
<tr>
<td>P120ΔΔ821-981</td>
<td>0.1 4/4 21</td>
<td>0.04 6/4 15</td>
</tr>
<tr>
<td>None (mock infection)</td>
<td>&lt;0.5 ± 0.5 0/4 0/4</td>
<td>0/4 0/4</td>
</tr>
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</table>

$^a$ Transformation was monitored by infecting bone marrow cells with virus stocks of matched titer.

$^b$ Some of the cells were plated in agar, and visible colonies were scored 10 days later (38). The mean number of transformed colonies per 10$^5$ nucleated bone marrow cells ± the standard error of the mean is shown. Values indicated with a less-than symbol represent the minimum number of colonies that could have been detected in instances where no transformants were observed.

$^c$ Cells plated in liquid medium were scored as transformed when rapidly transformed pre-B cells filled the culture fluid. The day transformation occurred and the frequency of transformed dishes out of the total number of dishes evaluated is shown.

FIG. 1. Ab-MLV mutants lacking portions of the COOH terminus are expressed and active in NIH 3T3 cells. (A) Diagram of the v-Abl proteins encoded by the P120 wild-type virus and the different COOH-terminal mutants. The numbers above each diagram refer to amino acid numbers. Lysates prepared from NIH 3T3 cells infected with each of the viruses 48 h earlier were analyzed by Western blotting with an anti-Abl antibody (B) or an anti-phosphotyrosine antibody (C).

in the liquid assay, a property that can reflect both the frequency and growth rate of the transformed cells. In this assay a 10-fold dilution of wild-type virus delays transformation by 2 days, and a 100-fold dilution delays transformation by 3 to 4 days (L. Banovic and N. Rosenberg, unpublished data). In contrast, both the δ621-670 and δ668-819 mutants transformed pre-B cells nearly as well as wild-type virus. Taken together these data suggest that sequences within the last 160 amino acids are required for efficient transformation of pre-B cells but that those in the central portion of the COOH terminus, including those implicated in JAK interaction (7) and DNA binding (22), play a less critical role.

Signals to the c-fos promoter are largely intact in the COOH terminus truncation mutants. The serum-responsive element in the c-fos gene is one important downstream target activated in response to the v-Abl expression gene (33, 36). Earlier analyses revealed that a v-Abl protein lacking the full COOH terminus was reduced by about 50% in its ability to activate this element (51). To determine if differences in the ability to stimulate the c-fos promoter correlated with reduced lymphoid cell transformation, 293T cells were transfected with a c-fos reporter and constructs expressing the different mutants. The cells were harvested, and luciferase activity was measured. Activity was standardized by using expression of a control, Renilla luciferase plasmid (Fig. 2). As expected, P120 expression activated the c-fos reporter and constructs expressing the different mutants. The results suggest that deficient signaling to c-fos does not explain the requirement for the COOH terminus in pre-B-cell transformation.

Decreased tyrosine phosphorylation of p62Dok. Earlier experiments demonstrated that phosphorylation of 62-kDa proteins was reduced in rare pre-B-cell transformants expressing COOH-terminal truncation mutants. This deficit was linked to effects on p120Ras-Gap and could be complemented by Ras overexpression (30). Later experimentation revealed that the
p62Dok protein, an adaptor molecule that can bind p120Ras-Gap, is heavily tyrosine phosphorylated in v-Abl-transformed cells (53) and that tyrosine phosphorylation appears to enhance its ability to bind Ras-Gap and sequesters this negative regulatory protein away from Ras (3, 53). To determine if expression of the COOH-terminal truncation mutants affected tyrosine phosphorylation of p62Dok, the protein was immunoprecipitated from pre-B cells transformed with the different mutants. All of the transformants expressed similar levels of p62Dok (Fig. 3), and those expressing \( \Delta \beta \) and \( \Delta \alpha \) expressed the same level of tyrosine phosphorylation as cells expressing P120. However, densitometric analysis revealed that cells expressing the \( \Delta \Delta \) v-Abl protein and those expressing \( \Delta \gamma \) displayed about twofold lower levels of tyrosine phosphorylation. Consistent with this pattern, a twofold reduction in the amount of p120Ras-Gap was recovered in these immune complexes. These data suggest that truncation of the COOH terminus of v-Abl leads to deficiencies in phosphorylation of p62Dok. These deficits may lead to suppression of Ras function, thus blunting the transforming response.

Reduced levels of Ras-GTP in carboxyl-terminal truncation and deletion mutants. Coinfection of bone marrow cells with a virus expressing v-Ha-Ras and COOH-terminal truncation mutants restores transforming activity (30), suggesting that one function of the COOH terminus involves signaling to Ras, a required intermediate in the v-Abl transformation pathway (42). To determine if v-Abl proteins containing different amounts of COOH-terminal sequence activate Ras, 293T cells were transfected with DNAs encoding the different v-Abl proteins and a c-Ha-Ras expression plasmid. The cells were serum-starved for 24 h and lysates were prepared. The level of activated Ras was assessed by using a GST-RBD fusion protein (19). This protein contains the Ras binding domain of Raf, a region that preferentially binds to Ras-GTP. The highest levels of binding were recovered from extracts expressing wild-type P120 and \( \Delta \gamma \) v-Abl proteins were deficient in stimulating Ras activity. However, the \( \Delta \gamma \) v-Abl protein also stimulated Ras to a lesser extent than the P120 protein. These data suggest that loss of COOH-terminal sequences affects the ability of v-Abl proteins to activate Ras but that the relationship of this feature to transfor-
mation is complex and that other sequences present in the COOH terminus can compensate for the deficiencies in Ras signaling observed for the Δ621-670 v-Abl protein.

Changes at the extreme COOH terminus compromise transformation. Analyses of the COOH-terminal truncation mutants highlighted the role of the COOH-terminal 160 amino acids in lymphoid transformation. One function attributed to the extreme COOH terminus of c-Abl and Bcr/Abl proteins involves interactions with G- and F-actins (26, 48, 50). Regions that interact with these molecules are deleted in both the Δ821-981 and ΔΔ mutants. To test the possibility that reduced transformation reflected loss of these motifs, three additional mutants were constructed (Fig. 5A). The P120Δ977-981 mutant encodes a v-Abl protein lacking the final 5 amino acids of v-Abl, residues required for strong F-actin association by c-Abl and Bcr/Abl (26). The P120Δ819-923 and P120Δ923-981 mutants express v-Abl proteins that retain a portion of the sequence missing in the Δ821-981 mutant; the v-Abl protein expressed by the Δ819-923 mutant is missing the majority of the G-actin binding domain, and the Δ923-981 mutant expresses a v-Abl protein that is missing the F-actin binding domain. Both the Δ819-923 and Δ923-981 mutants transformed NIH 3T3 cells, expressed proteins of the expected size, and induced elevated levels of phosphotyrosine (Fig. 5B).

However, the Δ977-981 mutant failed to transform NIH 3T3 cells, and Western analysis of the cells revealed that lower levels of the protein were expressed (data not shown). Expression of Δ977-981 was also examined with pre-B cells by using the superinfectable Ab-MLV–P70-transformed 7C411 cell line (12). Because the 7C411 cells are transformed with the P70/H590 strain of Ab-MLV (12), the presence of the second v-Abl protein can be detected with SDS-PAGE by its size. Cells were infected with the P120 wild-type strain or Δ977-981 expressed from the pSRαMSVtkneo vector that also expresses a neomycin resistance cassette (28), and populations carrying these viruses were selected by using G418 treatment. In comparison to cells superinfected with wild-type virus, cells infected with the Δ977-981 mutant expressed very low levels of this v-Abl protein (Fig. 5C).

Bone marrow cells were infected with each of the mutants and were monitored for transformation by using agar and liquid assays (Table 2). The Δ819-923 mutant retained the ability to transform lymphoid cells, although the frequency was low and transformation was delayed in the liquid assay. This delay most likely reflects the small number of transformed cells in each of the cultures, but it may also suggest that the transformants expand more slowly. The Δ977-981 mutant failed to transform pre-B cells even in the liquid assay, and only a fraction of liquid cultures infected with the Δ923-981 mutant became transformed. In this latter case, the time required for transformed cells to emerge was greatly extended.

New variants arise in pre-B cells infected with Δ923-981. Although most carboxyl-terminal truncation mutants transform pre-B cells poorly, the few transformants that arise almost always express the v-Abl protein encoded by the original mutant (30, 41). Similar results were obtained with cultures infected with the ΔΔ, Δ668-819, and Δ819-923 mutants (Fig. 6A and data not shown). However, analysis of the v-Abl proteins expressed by the transformants that arose in bone marrow cultures infected with Δ923-981 revealed the presence of...
v-Abl proteins that were smaller than those encoded by Δ923-981 (Fig. 6A). To determine if the smaller protein sizes reflected a stable change in the virus, NIH 3T3 cells were infected with filtered culture fluid harvested from the transformed pre-B cells. The infected NIH 3T3 cells displayed the typical morphology of Ab-MLV-transformed cells and in all cases expressed v-Abl proteins that were different in size from those of Δ923-981 but similar to those found in the original transformed pre-B cells (Fig. 6B).

The stable expression of v-Abl proteins of altered size strongly suggested that an additional mutation(s) had arisen in the pre-B cells infected with the Δ923-981 mutant. To test this possibility, DNA was prepared from three of the different isolates and the region encoding the COOH terminus was amplified by using PCR. In each case a mutation was detected that altered the reading frame in a fashion consistent with the new protein size (Fig. 6C). The presence of the original Δ923-981 deletion was also confirmed, indicating that the viruses arose by further mutation of the virus used to infect the pre-B cells. These data suggest that the Δ923-981 v-Abl protein is highly compromised in its ability to transform cells and that variants which correct this defect have a strong selective advantage. In addition, because all of these variants contain mutations that cause loss of additional amino acids, it appears that the precise structure of the extreme COOH terminus affects the ability of the protein to transform cells.

The extreme COOH terminus is important for v-Abl protein stability. Steady-state levels of the Δ977-981 v-Abl protein are low, and the Δ923-981 mutant fails to transform lymphoid cells unless additional mutation occurs. These data suggest that the nature of the extreme COOH terminus of the v-Abl protein may affect its stability in pre-B cells. To test this possibility, 7C411 cells (12) that had been superinfected with the Δ977-981 or Δ923-981 mutant or P120 wild-type virus were prepared. These superinfected 7C411 cells were used to compare the half-life of the P120 and Δ977-981 proteins by pulse-chase experiments with [35S]methionine-labeled cells. This analysis revealed that the half-life of the Δ977-981 protein was about 40 min, significantly less than the 190-min half-life observed for the P120 and P70 proteins (Fig. 7A). Precise quantitation of the half-life of the Δ923-981 mutant was difficult, because a variant appeared in 7C411 cells infected with this mutant while the newly infected cells were expanding. This variant became the most prominent newly expressed v-Ab protein in the cells (Fig. 7B). The half-life of the Δ923-981 protein appeared to be shorter than that of the P120, P70, or newly expressed v-Ab protein (data not shown). In addition, treatment of these cells with the proteosome inhibitor lactacystin revealed that levels of the Δ923-981 v-Ab protein were increased when proteosome function was blocked. Densitometric analysis demonstrated a four- to fivefold increase in the amount of Δ923-981 after 5 h of exposure to the drug; levels of the other v-Ab proteins expressed by the cells remained constant throughout the experiment (Fig. 7B). Taken together these data suggest that the structure of the extreme COOH terminus influences the ability of v-Ab proteins to transform cells by affecting protein stability. In addition, the loss of a relatively small portion of the extreme COOH terminus renders the protein unstable, a feature that can be overcome by removal of additional sequence in the region.

**DISCUSSION**

Our results demonstrate that the central portion of the v-Ab COOH terminus is largely dispensable for lymphoid transformation. The P120Δ668-819 mutant retains transforming potency that is close to that of wild-type pre-B cell, but it is missing the entire DNA binding domain (22), the JAK1 binding region (7), and PXXP motifs that could provide sites of protein-protein interaction. This picture contrasts sharply with the observation that mutants lacking the extreme COOH-terminal amino acids are significantly impaired for lymphoid transformation. Thus, our data define the sequences at the extreme COOH terminus as one region that is important for lymphoid cell transformation. These mutants have a complex phenotype, with some changes rendering the v-Ab protein unstable in lymphoid cells. However, instability alone does not account for the phenotype of all of the mutants, suggesting that motifs known to lie within this region play an important role in transformation.

The extreme COOH terminus of Ab proteins contains G- and F-actin binding regions that mediate functionally important interactions between both Bcr/Ab and c-Ab and the cytoskeleton (17, 26, 36, 48, 52). v-Ab protein has been found localized to focal adhesions (37), suggesting that contacts with cytoskeletal elements are made by v-Ab in transformed cells. However, the functional significance of cytoskeletal interactions for v-Ab-mediated transformation has received very limited attention. NIH 3T3 cells transformed by COOH-terminal truncation mutants and wild-type virus both display the characteristic rounded morphology and loosely adherent phenotype associated with v-Ab-mediated transformation, demonstrating that the presence of COOH-terminal sequences is not required for the reorganization that confers these properties. Nonetheless, the observation that both the Δ821-923 and Δ923-981 mutants, which encode v-Ab proteins that lack G- and F-actin binding regions, are highly compromised for lym-

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*a Transformation was monitored by infecting bone marrow cells with virus stocks of matched titer.
*b Some of the cells were plated in agar, and visible colonies were scored 10 days later (38). The mean number of transformed colonies per 10⁶ nucleated bone marrow cells ± the standard error of the mean is shown. Values indicated with a less-than symbol represent the minimum number of colonies that could have been detected in instances where no transformants were observed.
*c Cells plated in liquid medium were scored as transformed when rapidly transformed pre-B cells filled the culture fluid. The day transformation was noted and the frequency of transformed dishes out of the total number of dishes evaluated is shown.
*d NT, not tested.
phoid cell transformation coupled with the idea that these regions may facilitate cooperative binding to the cytoskeleton (48) could indicate that this interaction plays an important role in pre-B-cell transformation.

Other motifs within the final 160 amino acids could also be important. This portion of the v-Abl protein contains a site of PKC phosphorylation (31). However, mutations changing the serines within the COOH terminus that are targeted by PKC to alanines have no effect on the ability of v-Abl to transform pre-B cells (unpublished data). Thus, this type of modification does not appear to be important for v-Abl activity. In addition, in contrast to the PXXP motifs that are proximal to the SH1 domain, none of the PXXP motifs within this region have been shown to interact with other signaling molecules, raising the possibility that these are not functionally important for transformation.

The CTD-ID, a region highly conserved in c-Abl and the related c-Arg protein that interacts with RNA polymerase II (1), is found within the extreme COOH terminus. This region is interrupted in the v-Abl proteins encoded by both the Δ821-923 and Δ923-981 mutants. Although the cytoplasmic and plasma membrane localization of v-Abl suggests that it does not interact with RNA polymerase II, this region may serve as a docking site for other proteins. Activation of Nck is associated with the extreme COOH terminus of c-Abl, and that interaction appears to be indirect (46). Thus, another as-yet unknown protein could bridge between the CTD-ID and Nck and could transmit proliferative signals downstream.

An intact v-Abl COOH terminus appears to influence the ability of the protein to stimulate Ras. These interactions are reflected in the ability of the Δ668-820 v-Abl protein to both tyrosine phosphorylate p62Dok and to stimulate Ras activity.
FIG. 7. Changes at the extreme COOH terminus alter v-Abl protein stability. (A) 7C411 cells superinfected with either P120 wild-type virus or the Δ977-981 mutant were pulsed with [35S]methionine (at time 0); samples were removed after various minutes of chase as indicated by the numbers above the lanes and were analyzed by SDS-PAGE. The positions of the P70 protein and its glycosylated form (gP70) along with that of the v-Abl proteins encoded by the superinfecting viruses is indicated. (B) 7C411 cells were superinfected with either P120 wild-type virus or the Δ923-981 mutant. The cells were treated with 10 μM lactacystin for the indicated times, and lysates were analyzed by Western blotting using an anti-Abl antibody. The asterisk indicates the position of the variant formed in 7C411 cells infected with Δ923-981.

Both of these features are compromised in the COOH-terminal truncation mutants that are highly deficient in pre-B-cell transformation. This observation is consistent with the ability of Ras to complement the defect of similar mutants in lymho-3T3 cells, where transformation is accompanied by normal expression of the protein, and in pre-B cells, where the few transformants that arise contain new mutants, suggests that there are important differences in the way v-Abl protein expression is regulated in the two settings. A similar phenotype was noted with mutants that alter the Gag portion of v-Abl (32). Whether the rapid turnover of v-Abl proteins missing a portion of p12Gag-derived residues reflects a defect that is in some way related to that revealed by the COOH-terminal truncation mutants requires additional work. While misfolding could be the underlying cause, the observation that v-Abl protein instability occurs in pre-B cells suggests that an additional host protein(s) that does not regulate v-Abl in NIH 3T3 cells is involved. Because mutations that further truncate the v-Abl protein correct the deficiency in stability but do not fully restore transforming potential, it is likely that the COOH terminus influences lymphoid transformation in a complex fashion and that several different regions of the protein play important roles in the response. Understanding this complex process is likely to reveal important insights into the mechanism by which Abl oncogenes transform cells.

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REFERENCES


