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Activation of murine c-*abl* protooncogene: Effect of a point mutation on oncogenic activation

(Abelson leukemia virus/Hardy–Zuckerman feline sarcoma virus/protein kinase)

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ABSTRACT **Activation of the c-*abl* protooncogene occurs in Abelson murine leukemia virus, in Hardy–Zuckerman 2 feline sarcoma virus, and during the chromosomal translocations that generate *BCR-ABL* gene fusion products. To study the molecular mechanism involved in the c-*abl* activation, we have created a series of modifications in murine c-*abl* and assayed these constructs for oncogenic activity using the NIH 3T3 cell transformation assay. Our results show that amino-terminal deletions are sufficient for oncogenic activation of c-*abl* and high levels of oncogenic activities were generated by a deletion of 114 codons from the 5′ end that deleted the SH3 region. A deletion of 53 codons from the 5′ end (inclusive of deletions seen in Hardy–Zuckerman 2 feline sarcoma virus and *BCR-ABL* gene products) that retains the SH3 region of c-*abl* resulted in the generation of low levels of transforming activity. This transforming potential was substantially increased with the introduction of a G → A point mutation in codon 832 that is present in v-*abl*. The point mutation was found to affect the secondary structure and the tyrosine kinase activity of the mutant gene products.**

The v-*abl* gene was first identified as the transforming component of the Abelson murine leukemia virus (Ab-MuLV), a murine retrovirus that causes B-cell leukemia in mice. Several different isolates of Ab-MuLV have been identified, and two of them, termed the P120 and P160 strains, have been extensively studied during the past few years. More recently, a second retrovirus, termed Hardy–Zuckerman 2 feline sarcoma virus (HZ2-FeSV), was also found to have transduced the feline *abl* gene in the generation of an acute transforming virus. In addition to its activation by viral transduction, the *ABL* gene is also activated in Philadelphia chromosome-positive (Phi$^+$) human chronic myelogenous leukemias (CMLs) and some forms of Phi$^+$ acute lymphocytic leukemias (ALLs), which are characterized by a reciprocal translocation between chromosomes 9 and 22. This translocation results in the fusion of two cellular genes, the *ABL* gene and the *BCR* gene, resulting in the formation of a chimeric gene termed *BCR-ABL* (reviewed in refs. 1 and 2).

A comparative study of retroviral oncogenes and their cellular homologues has provided important information regarding the structural changes that protooncogenes undergo during the acquisition of transforming potential. Toward this goal, we had earlier reported the cloning and sequence analysis of Ab-MuLV proviral genome (P120 strain) as well as the murine c-*abl* cDNA (3, 4). A comparison of the nucleotide sequence of c-*abl* with its activated counterparts, P160 and P120, showed that both forms of the v-*abl* gene have suffered a loss of their 5′ sequences where 114 codons derived from the 5′ end of c-*abl* have been replaced by 240 codons derived from the *gag* gene (4, 5). In addition to the 5′ deletion, the P120 strain has undergone an internal in-frame deletion of 789 base pairs (bp) (263 codons) and a single point mutation (G → A) immediately downstream of the deletion [codon 832 of c-*abl* (type I) cDNA used in these studies] (4). Thus three structural changes, two deletions and a point mutation, appear to have accompanied the formation of the P120 genome. The P160 genome has a very similar structure to that of P120 but lacks the internal deletion seen in P120 (6). Earlier sequence studies had indicated the presence of a frame-shift mutation in the P160 genome in the region that is deleted in the P120 genome (5). To confirm this observation, we resequenced the P160 DNA clone used in these studies, and our sequence data (unpublished data) indicated that this frame-shift mutation is not present in the P160 clone that is used here, suggesting that this frame-shift mutation seen is either a sequencing error or a mutation that occurs in certain DNA clones of P160 but does not contribute to the transforming potential of the P160 genome. Thus, the P160 genome has undergone two alterations during its oncogenic activation, one of which is a deletion at the 5′ end and the other a point mutation (at position 832 of the corresponding c-*abl* cDNA clone) of the encoded protein.

The HZ2-FeSV genome has been found to have suffered both 5′ and 3′ deletions, where a stretch of 51 codons derived from the 5′ end has been replaced by a stretch of 344 codons of the viral *gag* gene and a stretch of 633 codons at the 3′ end has been replaced by 200 codons derived from the *pol* gene of the helper virus (7). As the feline c-*abl* gene sequence has not been determined, it is at present unclear as to whether the feline v-*abl* gene has undergone any point mutations in addition to these gross deletions.

The activation of the human *ABL* gene in Phi$^+$ CML occurs as a result of chromosomal translocation between chromosomes 9 and 22 resulting in the replacement of 26 codons derived from the 5′ end of the *ABL* gene by 927 codons derived from the *BCR* gene (8, 9). Sequence analysis of a cDNA clone derived from the K562 cell line indicates there are no additional mutations in the c-*abl* coding portion of this gene (10). Thus, in all the three systems, the activation of the c-*abl* gene was found to be associated with the deletion of a varying proportion of 5′ coding sequences derived from exons 1 and 2 of the c-*abl* gene and their replacement with either viral or *BCR* sequences.

An important biological consequence of these deletions and mutations is seen in their relative transforming potential. While the Ab-MuLV and HZ2-FeSV gene products have been known to be potent transforming agents (7, 11), the *BCR-ABL* gene product is weakly transforming (12), being

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Abbreviations: Ab-MuLV, Abelson murine leukemia virus; Mo-MuLV, Moloney murine leukemia virus; HZ2-FeSV, Hardy–Zuckerman 2 feline sarcoma virus; Phi$^+$, Philadelphia chromosome-positive; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia.

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unable to transform NIH 3T3 cells *in vitro* (13). This observed difference in transforming activity could be due to one or all of the three structural differences observed between the three activated forms of *abl*. (*i*) The amino-terminal end of the viral *gag* gene contains the site for myristoylation that allows efficient localization of the transforming protein to the membrane. (*ii*) Deletions and point mutation seen in the v-*abl* gene that are not seen in the *BCR-ABL* and c-*abl* gene products contribute to the oncogenic potential of the v-*abl* gene product. (*iii*) The extent of amino-terminal truncation (26 vs. 51 vs. 114 amino acids) seen between the three activated forms contributes to the varying degree of transforming activity seen with the three gene products. To discern between these alternatives, we systematically attempted to create deletions and mutations in the c-*abl* coding sequences and assessed their effect on the transforming activity of the resulting chimeric genes. Our results suggest that activation occurs with a deletion of 5′ *abl* sequences and their replacement with *gag* sequences, whereas the point mutation observed in the v-*abl* gene enhances the transforming potential of c-*abl* with an altered 5′ end.

MATERIALS AND METHODS

Cells and DNA Transfection Assays. NIH 3T3 cells were grown in Dulbecco's modified Eagle's medium containing 10% calf serum (GIBCO) and antibiotics. One hundred nanograms of linearized plasmid DNA was transfected as described (14). Individual foci were cloned by glass ring trypsinization and expanded for analysis, or G418-resistant colonies were mass cultured.

***abl* Plasmid Construction.** c-*abl* type I (4), Ab-MuLV P120 (3), Ab-MuLV P160 (6), and the retroviral vector pMV7 (15) were used for the construction of various mutants described.

The complete c-*abl* (type I) reading frame was inserted into the vector pMV7 by digestion with *Eco*RI and *Bam*HI followed by blunting with the Klenow fragment of DNA polymerase I. A vector derived from Ab-MuLV proviral genome (pC-1) was constructed by digestion with *Bst*EII and *Hin*dIII, blunt-ending, and addition of *Eco*RI linkers. For the construction of *gag*-c-*abl* construct, this vector was digested with *Eco*RI and *Bam*HI, and the *Eco*RI to *Bam*HI fragment of c-*abl* (I) was inserted. This allowed in-frame fusion of the Moloney murine leukemia virus (Mo-MuLV) p16 *gag* gene with the c-*abl* coding sequences. The same gene coding for *gag*-c-*abl* fusion protein was inserted into pMV7 following digestion with *Pvu* I and *Bam*HI, blunt-ending, and ligation into the *Eco*RI site of the vector. Clone Δ61-c-*abl* has a 183-base 5′ deletion of c-*abl* and was constructed by the addition of an ATG-containing linker to a prematurely terminated cDNA clone and insertion of the resulting open reading frame into pMV7.

Ab-MuLV-based constructs were made using the viral promoter elements and the p15 *gag* ATG initiation codon present in the pC-1 vector. Chimeras between v-*abl* and c-*abl* clones were constructed by reciprocal exchange of fragments generated by digestion of the cloned DNAs with *Bsm* I and *Bam*HI.

Immunoprecipitation and Kinase Assay. Immunoprecipitation and kinase assays were performed as described by Konopka and Witte (16). The peptide-derived anti-v-*abl* antiserum was kindly provided by R. Arlinghaus and has been described (17).

RESULTS

Construction of Retroviral Vectors Coding for c-*abl*, v-*abl*, and Mutant Forms of c-*abl*. Construction of recombinants between v-*abl* and c-*abl* genes was carried out with the Ab-MuLV proviral DNA clone, such that the viral long terminal repeat structure and transcription and translation initiation sites are unaltered. When it was necessary to introduce a drug-resistance marker, the coding sequences were introduced into a murine retroviral vector, pMV7, which carries a selectable G418 drug-resistance marker (15). This vector, which is derived from Mo-MuLV and has a similar structure as that of Ab-MuLV, was previously shown to express v-*abl* and c-*abl* gene products efficiently and has been used by us and others in transformation assays (12, 13). Usage of these two vectors allowed us to directly test the transforming potential of the cloned viral DNAs in an NIH 3T3 assay and to rescue the transforming virus to test its ability to transform cells *in vitro* and *in vivo*. The presence of the drug-resistance marker in the pMV7 vector also allowed us to monitor the relative efficiency obtained with each transfection assay independent of focus formation, which was essential in the case of nontransforming constructs. The presence of *Xmn* I and *Bsm* I restriction enzyme cleavage sites at the 5′ end of c-*abl* cDNA clone provided us with a convenient way to create 5′ deletions and chimeric hybrids between v-*abl* and c-*abl* DNA clones. The structure of the various mutant forms of the *abl* gene studied in this communication is presented in Fig. 1.

Transforming Potential of c-*abl* and *gag-abl* Fusion Products. The transforming potential of the various mutant forms

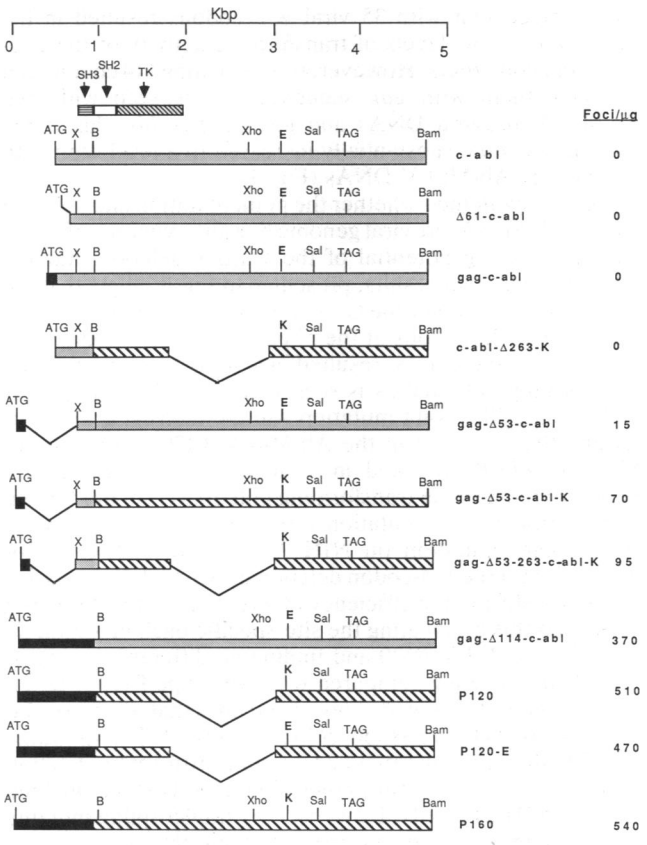

FIG. 1. Construction of retroviral vectors expressing various forms of *abl* gene. Details of vector construction are provided in the text. The SH3, SH2, and kinase regions are indicated. Clones of c-*abl* cDNA (type I), *gag*-c-*abl*, Δ61-c-*abl*, and c-*abl*-Δ263-K were inserted into the retroviral vector pMV7, whereas the remaining constructs were made in Ab-MuLV. Dark boxes represent viral *gag* sequences, hatched boxes represent v-*abl* sequences, and gray boxes represent the c-*abl* sequences. B, *Bsm* I; X, *Xmn* I; Xho, *Xho* I; Sal, *Sal* I; Bam, *Bam*HI. The location of the mutation is shown by the normal glutamic acid residue (E) or the mutant lysine amino acid (K), and the deletions are indicated by bent lines. One hundred nanograms of linearized proviral DNA was transfected onto NIH 3T3 cells, and the results have been multiplied by 10 and expressed as focus formation per μg of DNA. Kbp, kilobase pairs.

of the *abl* gene was tested by using the NIH 3T3 transformation assay, as described (14). In all instances, the transformation assays were carried out in the absence of any Mo-MuLV DNA so that the true transformation efficiency could be scored without the formation of any secondary foci due to the spread of the virus and to prevent recombination between the two DNAs. The transforming activities obtained with various constructs are presented in Fig. 1. The results show that a retroviral vector expressing c-*abl* alone was incapable of transforming NIH 3T3 cells. Similarly, deletion of 61 codons from the 5′ end (a stretch of deletion inclusive of the deletions seen in the HZ2-FeSV and *BCR-ABL* gene products) by itself had no effect on the transforming potential of c-*abl* as these DNA constructs also showed no focus formation in the NIH 3T3 assay. In addition, fusion of the complete c-*abl* coding sequences to *gag* sequences of Mo-MuLV also had no enhancing effect on the transforming potential of c-*abl*. Introduction of the internal deletion and point mutation present in the P120 genome without altering the 5′ end also had no activating effect on the c-*abl* genome.

We next tested the effect of 5′ deletions and their replacement with the viral *gag* sequences on the transforming potential of the c-*abl* gene. Thus a deletion of 53 codons from the 5′ end (comparable to the deletion seen in HZ2-FeSV and inclusive of the deletion seen in *BCR-ABL* gene products) and their replacement with 35 viral *gag* codons resulted in the generation of low levels of transforming activity of the *gag*-Δ53-c-*abl* constructs. However, by eliminating 114 codons and replacing them with *gag* sequences (as is seen with two Ab-MuLV proviral DNAs and *gag*-Δ114-c-*abl*), this transforming potential substantially increased to a level seen with the proviral Ab-MuLV DNAs (Fig. 1).

We next examined whether the point mutation and deletion seen in Ab-MuLV proviral genome had any synergic effect on the transforming potential of the various deletion mutants studied above. Our results, presented in Fig. 1, show that the simple introduction of the G → A point mutation in *gag*-Δ53-c-*abl* (where 53 codons at the 5′ end have been replaced by the viral *gag* sequences) resulted in a dramatic increase in its transforming potential as is seen with *gag*-Δ53-c-*abl*-K. Introduction of the point mutation and the internal deletion of codons 542–805 seen in the Ab-MuLV P120 genome (*gag*-Δ53-263-c-*abl*-K) resulted in a slight but not significantly higher increase in the transforming potential compared to the introduction of point mutation alone. Interestingly, the effect of the point mutation on NIH 3T3 transformation is not apparent with the 114-codon deletion of Ab-MuLV, as shown by the transformation efficiency of *gag*-Δ114-c-*abl* DNA. We confirmed this by creating the site-specific back mutation (A → G) in Ab-MuLV P120 and finding no difference in transformation efficiency compared with wild-type P120 (Fig. 1).

Fig. 2 shows the morphology of cell lines that were isolated from foci or G418-resistant colonies. NIH 3T3 cells transfected with c-*abl* constructs or its nontransforming deletion mutants showed a flat morphology and closely resembled the parental NIH 3T3 cells. However, the transforming mutants described in Fig. 1 generated cell lines that were highly refractile and spherical, as is seen with the two Ab-MuLV proviral DNAs. The only exception was the cells transformed by *gag*-Δ53-c-*abl* DNA, which were more fusiform and less refractile than the Ab-MuLV-transformed cell lines. Introduction of a point mutation in this DNA not only resulted in increased transforming potential of this DNA clone but also in the alteration of the morphology of the transformed cells into a more malignant phenotype.

Analysis of the Tyrosine Kinase Activity of the Chimeric *abl* Proteins. The *abl* gene products have earlier been shown to possess an associated tyrosine kinase activity that is substantially enhanced following its oncogenic activation (18–20). We, therefore, analyzed whether the variability in the transformation potential of various mutants of *abl* correlated with their kinase activity. It was especially interesting to see if the point mutation had a synergistic effect that correlated with its increased transforming activity. The results presented in Fig.

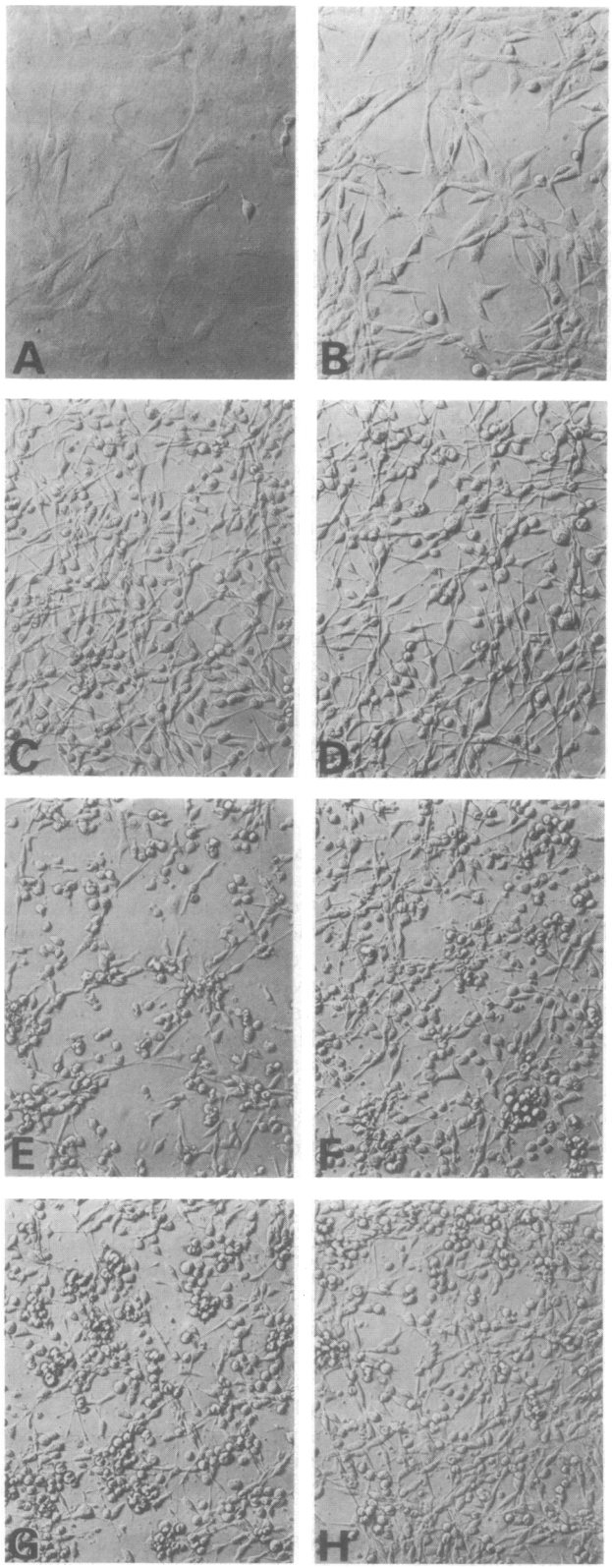

FIG. 2. Morphology of NIH 3T3 cells isolated following transfection with various *abl* constructs. (*A*) NIH 3T3. (*B*) Δ61-c-*abl*. (*C*) *gag*-Δ53-c-*abl*. (*D*) *gag*-Δ53-263-c-*abl*-K. (*E*) *gag*-Δ53-c-*abl*-K. (*F*) *gag*-Δ114-c-*abl*. (*G*) Ab-MuLV P120. (*H*) Ab-MuLV P160. (Photographed at ×200 with Hoffman modulation contrast.)

3 show that the cell lines transfected with the nontransforming constructs (lanes 2–5) failed to show any kinase activity in immune complex kinase assays (16). On the other hand, cell lines transfected with highly transforming mutants of *abl*, P120, P160, and *gag*-Δ114-c-*abl* constructs showed potent autophosphorylation as well as phosphorylation of cellular proteins (lanes 9–11). The constructs with lesser transforming activity, *gag*-Δ53-c-*abl*-K and *gag*-Δ53-263-c-*abl*-K (lanes 7 and 8), showed autokinase activity similar to the more potent transforming species. However, it is interesting to note that with these mutants the phosphorylation of non-*abl* proteins is considerably less compared to that seen with mutants with more potent transforming activity.

Cells transfected with *gag*-Δ53-c-*abl* constructs expressed a protein with extremely low kinase activity (Fig. 3, lane 6), which could only be seen with prolonged exposure (data not shown). However, the introduction of the point mutation at codon 832 of c-*abl* in this clone had a profound enhancing effect on its kinase activity (Fig. 3, lane 7), suggesting that enhanced transformed activity seen with this mutant correlated with its increased kinase activity.

To further confirm this observation, we next determined the ability of various mutants to phosphorylate an exogenous substrate, such as enolase (21). The results of this study presented in Fig. 4 demonstrate that neither NIH 3T3 cells (lane 1) nor the nontransforming mutant of c-*abl* (lane 2) was able to phosphorylate enolase. A very low level of enolase phosphorylation was observed with cell extracts transformed by *gag*-Δ53-c-*abl* (lane 3), which is also shown with prolonged exposure of the autoradiograms (lane 6). However, with the introduction of the point mutation or point mutation and deletion (lanes 4, 5, and 7) into these DNA clones, a dramatic increase in their kinase activity was observed. The levels of enolase phosphorylation observed closely correspond to the transforming activity exhibited by these various mutant DNAs, further supporting the observation that the point mutation seen in Ab-MuLV proviral DNA acts to increase the transforming potency by enhancing the kinase activity.

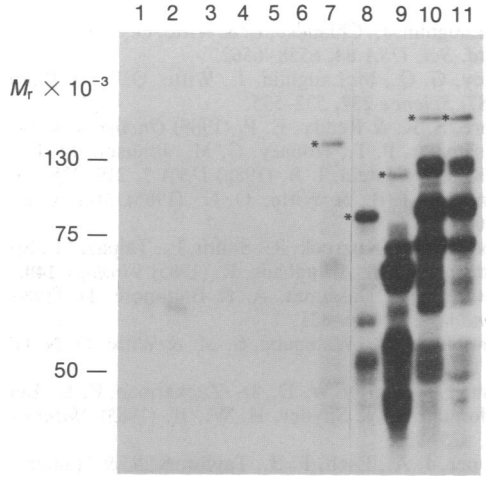

FIG. 3. Immune complex kinase assay of *abl* proteins in cell lysates from transfected NIH 3T3 cells (16). *abl* proteins from cell lysates containing equal amounts of protein were immunoprecipitated with α-v-*abl* TL037 antiserum and collected by the addition of protein A-Sepharose beads. Immune complexes were suspended in 15 μl of kinase buffer (20 mM Hepes, pH 7.0/0.1% Triton X-100/20 mM $MnCl_2$/150 mM NaCl) and labeled for 15 min at room temperature following the addition of 10 μCi (1 Ci = 37 GBq) of [γ-^{32}P]ATP in 3 μl of buffer. The proteins were resolved on a 6% SDS/PAGE gel and exposed to film for 2 min. The autophosphorylated *abl* proteins are indicated by the asterisks. Lane 1, NIH 3T3; lane 2, c-*abl*; lane 3, *gag*-c-*abl*; lane 4, c-*abl*-Δ263-K; lane 5, Δ61-c-*abl*; lane 6, *gag*-Δ53-c-*abl*; lane 7, *gag*-Δ53-c-*abl*-K (M_r 140,000); lane 8, *gag*-Δ53-263-c-*abl*-K (M_r 90,000); lane 9, P12 (M_r 120,000); lane 10, *gag*-Δ114-c-*abl* (M_r 160,000); and lane 11, p160 (M_r 160,000).

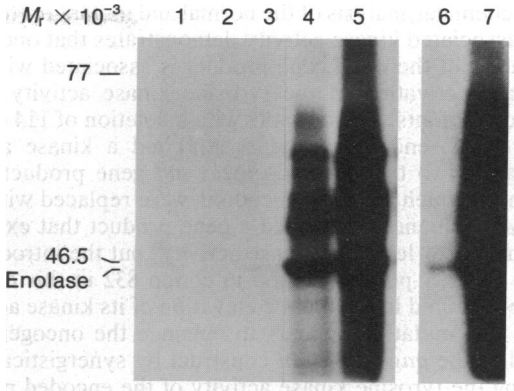

FIG. 4. Phosphorylation of acid-denatured enolase (20) by cell lysates from NIH 3T3 cells transfected with various DNA constructs. Cell lysates were equilibrated for protein concentration and immunoprecipitated with anti-v-*abl* TL037 antiserum. To the immune complex 10 μCi of [γ-^{32}P]ATP and 1 μl of rabbit muscle acid-denatured enolase were added. The reaction mixture was incubated for 15 min at room temperature and analyzed on a 6% SDS/PAGE gel. Autoradiography of the dried gel was for 20 min. Lane 1, NIH 3T3 cells; lane 2, Δ61-c-*abl*; lane 3, *gag*-Δ53-c-*abl*; lane 4, *gag*-Δ53-c-*abl*-K; and lane 5, *gag*-Δ53-263-c-*abl*-K. Lanes 6 and 7 are the same as lanes 3 and 4, but the exposure time was 40 min.

DISCUSSION

Results presented in this communication demonstrate that the oncogenic activation of the c-*abl* gene is influenced by the extent of amino-terminal deletions as well as by an internal point mutation. Thus, full oncogenic potential of this oncogene, as measured by NIH 3T3 transformation assay, can be achieved by a deletion of 114 codons from the 5′ end of the c-*abl* gene and their replacement by viral *gag* sequences. The 5′ end of the c-*abl* gene (see Fig. 1) has previously been shown to contain two regions of homology with the c-*src* sequences, which have been designated as SH3 and SH2 regions. Not only are these sequences present in all *src*-related kinases but also the SH3 domain has been found in phospholipase C, v-*crk*, GAP, α-spectrin, myosin I, and a yeast actin binding protein (reviewed in ref. 22; ref. 23). The SH3 region is thought to play the role of a negative regulatory element since the oncogenic activation of c-*src* has been observed following the introduction of point mutations in the SH3 region (24, 25). The presence of viral *gag* sequences in the mutants studied here seems to provide an important structural requirement—namely, the provision of a myristoylation signal that allows efficient membrane localization of the oncogenic product. This localization of the *abl* gene product has recently been demonstrated to be characteristic of the transforming protein (26).

Our results also demonstrate that when a limited set of sequences is deleted from the 5′ end of this gene, there is a considerable reduction in the transforming potential of the mutant gene. Thus, replacement of the first 53 codons with p15 viral *gag* sequences resulted in the generation of a low level of transforming activity. However, this transforming activity was considerably enhanced by the introduction of a G → A point mutation in codon 832 of the c-*abl* gene, a point mutation that results in the substitution of lysine for glutamic acid, and was originally seen by us in the v-*abl* gene sequences (4). Thus, this point mutation appears to have a synergistic effect on the oncogenic activation of this gene, especially in instances where only a limited set of sequences is deleted from the 5′ end of the gene.

Biochemical analysis of the normal and mutant proteins for their associated kinase activity demonstrates that oncogenic activation of the c-*abl* gene product is associated with concomitant activation of the tyrosine kinase activity of the deletion mutants. The mutants with a deletion of 114 codons from the 5′ end of the gene exhibited a kinase activity comparable to that of wild-type v-*abl* gene products. The mutant in which the first 53 codons were replaced with viral p15 *gag* sequences produced a gene product that exhibited extremely low levels of kinase activity, but the introduction of the G → A point mutation in codon 832 in this deletion mutant resulted in a dramatic elevation of its kinase activity. Thus, this mutation appears to enhance the oncogenic potential of the *gag*-Δ53-c-*abl* construct by synergistically enhancing the tyrosine kinase activity of the encoded protein.

The computer analysis of the c-*abl* amino acid sequence using the Chou–Fasman plot structure program for secondary structure (27) of gene products with and without the point mutation at codon 832 shows that this mutation could result in an alteration of the secondary structure, where the turn-helix-turn structure seen with the normal gene product is disrupted (data not shown). The synergistic effects seen between the 5′ deletions and internal point mutation in generating the transforming potential and tyrosine kinase activity of the protein products suggest a possible intramolecular interaction between these two domains—i.e., the SH3 domain and the domain encoded by the region that includes codon 832. It is possible that these two domains interact with each other in their native state bringing about an intramolecular negative regulatory effect on the protein molecule, and this interaction is disrupted either by extensive deletions in the 5′ end or a smaller deletion at the 5′ end and concomitant point mutation in codon 832. This turn-helix-turn structure is also conserved in the human ABL protein, indicating its functional importance (10).

While this work was in progress, two reports appeared where the effect of 5′ deletions and their replacement by viral *gag* sequences or deletions in the naturally myristoylated c-*abl* (type IV) were studied (28, 29). Our results agree with theirs with respect to lack of transformation by c-*abl* and myristoylated c-*abl* proteins. However, these reports suggest that complete deletion of the SH3 region is essential for the oncogenic activation of the c-*abl* gene. Our results differ from these two reports in that we observe oncogenic activation of the c-*abl* gene without deletion of the SH3 domain as is seen in the *gag*-Δ53-c-*abl*, *gag*-Δ53-c-*abl*-K, and *gag*-Δ53-263-c-*abl*-K constructs. Furthermore, these investigators have not studied the effects of point mutation in codon 832, which seems to impart an enhanced transforming activity over the weakly oncogenic *gag*-Δ53-c-*abl* gene product.

The results presented in this communication also have important implications for the oncogenic activation of human ABL in Phi$^+$ CML and Phi$^+$ ALL patients. The *BCR-ABL* gene has been shown to code for a protein in which the first 26 amino acids of the c-*abl* protein have been replaced by a stretch of amino acids derived from the *BCR* gene (8, 9). It is interesting to note that the onset of Philadelphia chromosome does not immediately result in the onset of CML or ALL. Patients who are Phi$^+$ live fairly normal lives for prolonged periods. However, an alteration in the nature of the disease occurs that seems to result from a single clone evolving into a more malignant cell type. At this acute phase of the illness, survival of the patient is limited to a few months (reviewed in ref. 30). The molecular events that dictate this transition are at present unclear. The results presented in this communication indicate why the *BCR-ABL* gene product, which has a minimal deletion of amino-terminal amino acids derived from the *ABL* gene, is weakly transforming or even nontransforming in NIH 3T3 cells (12, 13). Additional accumulation of mutations in functional domains such as that in the vicinity of codon 832 are likely to potentiate its transforming activity. It is possible that such mutations constitute a secondary event, at least in some cases of CML and ALL, that leads to a more malignant disease. A careful sequence analysis of the *BCR-ABL* gene product from a large population of Phi$^+$ CML and ALL patients in blast and chronic phases of the disease should clarify the nature of molecular events that lead to the oncogenic activation of this gene in human leukemias.

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