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Phosphorylation of the Abelson Murine Leukemia Virus Transforming Protein

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The Abelson murine leukemia virus transforming gene product is a phosphorylated protein encoded by both viral and cellular sequences. This gene product has an amino-terminal region derived from the *gag* gene of its parent virus and a carboxyl-terminal region (*abl*) derived from a normal murine cellular gene. Using a combination of partial proteolytic cleavage techniques and antisera specific for *gag* and *abl* sequences, we mapped in vivo phosphorylation sites to different regions of the protein. Phosphoproteins encoded by strain variants and transformation-defective mutants of Abelson murine leukemia virus with defined deletions in the primary sequence of the *abl* region were compared by two-dimensional limit digest peptide mapping. Specific phosphorylation pattern differences for wild-type and mutant proteins probably represented deletions of specific phosphate acceptor sites in the *abl* region. An in vitro autophosphorylation activity copurified with the Abelson murine leukemia virus protein from transformation-competent strains. A peptide analysis of such in vitro reactions demonstrated that these phosphorylation sites were restricted to the amino-terminal region, and the specific sites appeared to be unrelated to the sites found on proteins phosphorylated in vivo. Thus, the autophosphorylation reaction probably correlates with an activity important in transformation, but the specific end product in vitro bears little resemblance to its function in vivo.

Abelson murine leukemia virus (A-MuLV) is a member of an increasingly large group of replication-defective rapidly transforming retroviruses (9, 27). Derived from the replication-competent Moloney murine leukemia virus by recombination with a normal cellular gene, A-MuLV expresses transforming activity for both established fibroblast lines in vitro and pre-B cells in vitro and in vivo (1, 2, 17-19, 24). A-MuLV retains homology to Moloney murine leukemia virus at the ends of its genome but contains sequences derived from a cellular gene internally (7, 15, 26, 31, 32). These internal sequences are referred to as the *abl* region. The nucleic acid recombination in A-MuLV results in the production of a fused protein containing an amino-terminal region precisely homologous to Moloney murine leukemia virus *gag* gene subregions p15 and p12 and a portion of p30 and a carboxyl-terminal region derived from the acquired cellular sequences. An auto-phosphorylation activity specific for tyrosine residues copurifies with the A-MuLV protein (28). No other A-MuLV-specific primary translation product has been identified. Deletion and premature termination mutants in the carboxyl-terminal *abl* region which show parallel changes in transforming potential and the level of the in vitro phosphotyrosine kinase activity (20, 21, 23, 29) strongly suggest that this protein contains the transforming function for A-MuLV.

The A-MuLV protein is a phosphoprotein in vivo (16, 32). Defining the role of the cellular protein kinases or the auto-phosphorylation activity of the A-MuLV protein in these in vivo post-translational modifications could aid in our understanding of the control of the function of this protein. In this study we began such an analysis by identifying the regions and specific sites on the A-MuLV protein which are phosphorylated, as well as the specific differences expressed by mutant strains of A-MuLV.

MATERIALS AND METHODS

Cells and viruses. Each strain of A-MuLV was isolated in a transformed nonproducer clone of NIH/3T3 cells (20, 21, 24).

Labeling and immunoprecipitation. The tech-

niques used for metabolic labeling with [^{35}S]methionine, in vitro auto-phosphorylation with [γ-^{32}P]ATP, and in vivo labeling with $^{32}P_i$ have been described previously (20, 28, 32). The methods used for immunoprecipitation of detergent-lysed cellular extracts with *gag* gene-reactive antiserum (goat anti-Moloney virions, rabbit anti-p15, and goat anti-p12) and *abl*-specific anti-Abelson tumor serum (anti-AbT) have also been described previously (11, 30, 32). Samples were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and fluorography (3, 13).

Partial proteolytic one-dimensional peptide mapping. A modification of the method of Cleveland et al. (4, 34) was used. Samples isolated by immunoprecipitation with a standardized amount of each specific antiserum were recovered from the *Staphylococcus aureus* immunoadsorbant in a solution containing 1% SDS, 1% 2-mercaptoethanol, and 50 mM Tris (pH 6.8). A portion of each sample (usually 10 μl) was mixed with an equal volume of enzyme solution in 50 mM Tris (pH 6.8) and then incubated at 37°C (for times, see below). The reaction was terminated by adding an equal volume of 2% SDS–2% 2-mercaptoethanol and heating at 70°C for 5 to 10 min. Digested or control preparations were analyzed directly by SDS-polyacrylamide gel electrophoresis. Alternatively, preparations were diluted into immunoprecipitation detergent lysis buffer, and fragments were immunoprecipitated with antisera specific for regions of the A-MuLV protein.

Two-dimensional peptide mapping. Different A-MuLV proteins labeled with ^{32}P from in vitro kinase reactions or in vivo labeling were isolated by immunoprecipitation and gel electrophoresis, visualized by autoradiography, and excised from dried gels. Gel pieces were processed and limit digested with α-chymotrypsin as previously described (5). Samples were electrophoresed in the first dimension on thin-layer cellulose sheets in formic acid–acetic acid–water (5:15:80) and then chromatographed in the second dimension by ascending chromatography in acetic acid–pyridine–butanol–water (5:25:32.5:20) containing 7.2% PPO (2,5-diphenyloxazole; Eastman Chemicals), as previously described (5, 6).

RESULTS

Summary of A-MuLV strains. Table 1 shows summary of the genome structures, transforming activities, and relative in vitro tyrosine auto-phosphorylation activities of the A-MuLV strains used in this study. Each A-MuLV protein and virus strain is referred to by the size of the protein in kilodaltons, as estimated by SDS-polyacrylamide gel electrophoresis.

The relative genome structures deduced from Southern blot analyses (7, 8) demonstrated that the strain P160 genome (6.3 kilobases) contained a region of 800 bases in the *abl*-specific region that was not found in strain P120 or the other strains. The genomes of strains P120, P100, P90, and P85 were the same size (5.5 kilobases), and the smaller primary translation products of these strains were probably due to premature stop codons caused by single base changes or very small deletions. Strain P92 was a true transformation-defective mutant. Its genome had an additional deletion of 700 bases in the center of the *abl* sequences, which resulted in a total loss of transforming activity for fibroblasts and lymphoid cells and a loss of in vitro kinase activity (29).

All strains appeared to have identical Moloney murine leukemia virus-derived *gag* gene sequences at the 5′ ends of their genomes and to share extensive regions of the *abl* sequences. These observations were supported by immunoprecipitation analyses in which we used rabbit monospecific antisera against the *gag* gene products p15, p12, and p30 and a tumor regressor serum (anti-AbT) specific for the *abl*-encoded region (20, 21, 30).

Limited proteolysis of A-MuLV proteins. From the nucleic acid structures of the different A-MuLV strains, we predicted a common amino-terminal region of about 50,000 daltons for all of the A-MuLV proteins (comprised of *gag* gene and some *abl* gene sequences) and carboxy-terminal regions of varying lengths and compositions (derived entirely from *abl* sequences).

We used partial proteolytic cleavage (Fig. 1) in the presence of SDS and an analysis of the resulting fragments by gel electrophoresis as described by Cleveland et al. (4, 34) to compare proteins with homologous regions as well as unique sequence regions. To begin such an analysis, we suspended [^{35}S]methionine-labeled P90 (Fig. 1, lanes A), P120 (lanes B), and P160 (lanes C) proteins isolated from transformed nonpro-

TABLE 1. *Summary of A-MuLV strains*

Strain[a]	Complete genome size (kilobases)	*abl* region size (kilobases)	Transformation activity		
			Fibroblast	Lymphoid	In vitro kinase
P160	6.3	4.3	+++	+++	+++
P120[b]	5.5	3.5	+++	+++	+++
P100[c]	5.5	3.5	+++	+	+
P90[c]	5.5	3.5	+++	+	+
P85[c]	5.5	3.5	+++	+	+
P92[d]	4.8	2.8	−	−	−

[a] Each strain of A-MuLV was named for the size of its encoded protein in kilodaltons.

[b] Strain P120 was derived by deletion of 800 bases from the center of the *abl* sequences in strain P160.

[c] Strains P100, P90, and P85 were derived from strain P120 and probably represented chain termination mutants caused by point mutations or small deletions.

[d] Strain P92 was derived from strain P120 by a 700-base deletion from the *abl* sequences.

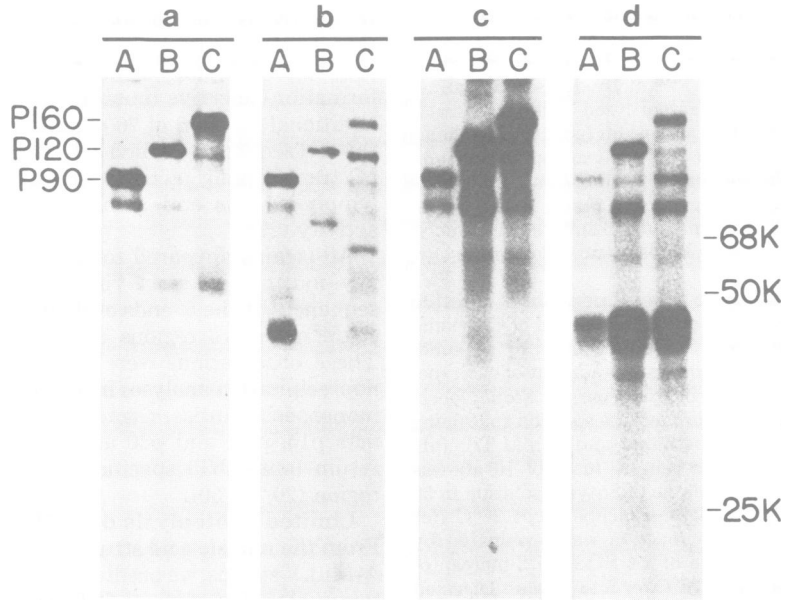

FIG. 1. *Partial proteolytic cleavage of A-MuLV proteins. (a and b) Transformed nonproducer fibroblast clones of strains P90 (lanes A), P120 (lanes B), and P160 (lanes C) were labeled for 2 h with [^{35}S]methionine (100 μCi/5 × 10^6 cells), extracted, clarified, and immunoprecipitated with goat anti-Moloney virion antiserum (32). (a) A portion of each preparation was denatured, electrophoresed on a 10% polyacrylamide gel, and developed by fluorography (3, 13). (b) Equivalent portions (representing 2.0 μl of goat antiserum) were denatured in SDS gel sample buffer (10 μl), diluted with 10 μl of S. aureus V8 protease (10 μg/ml) in 50 mM Tris (pH 6.8), and reacted at 37°C for 30 min. The reactions were terminated by adding 10 μl of twofold-concentrated gel sample buffer and heating at 70°C for 10 min; the resulting preparations were then analyzed as described above. (c and d) Equal numbers of unlabeled cells were extracted, clarified, and immunoprecipitated as described above; then, the A-MuLV proteins were labeled with [γ-^{32}P]ATP by the auto-phosphorylation reaction (28). Samples were treated as described above for control (c) and protease-digested (d) comparisons. The positions of protein molecular weight markers, including bovine serum albumin (molecular weight, 68,000 [68K]), heavy-chain immunoglobulin (50,000), and light-chain immunoglobulin (25,000), are also shown.*

ducer NIH/3T3 fibroblast clones by immunoprecipitation in 0.5% SDS buffer and subjected them to control conditions (Fig. 1a) or to digestion with *S. aureus* V8 protease (Fig. 1b). These preparations (Fig. 1a) contained small amounts of a common, naturally occurring fragment of 80,000 daltons, as well as the expected initial 90,000-, 120,000-, and 160,000-dalton proteins of the different A-MuLV strains. Each cleaved preparation (Fig. 1b) contained some residual starting material and a distinctive cleavage fragment, which varied in size from 45,000 daltons (Fig. 1b, lane A) to 75,000 daltons (lane B) to 115,000 daltons (lane C). These cleavage fragments would have been expected if a major site about 45,000 daltons from the amino terminus of each protein had been cleaved, generating a common amino-terminal fragment and different carboxyl-terminal fragments from the different A-MuLV strains. Longer exposures of such cleavage patterns of [^{35}S]methionine-labeled A-MuLV proteins did show the predicted common 45,000-dalton fragment. This amino-terminal region was relatively methionine poor and was documented better with other labeling techniques.

The same A-MuLV proteins (P90, P120, and P160) were isolated by immunoprecipitation, labeled in vitro with [γ-^{32}P]ATP by the tyrosine auto-phosphorylation reaction (28), and subjected to the same digestion protocol (Fig. 1c and d). All three strains showed as major cleavage products a set of fragments of about 45,000 daltons, which could have represented the predicted common amino-terminal region. Time course or dose-response digestion studies showed that the uppermost bands in the 45,000-dalton region appeared first and were probably then cleaved to yield the slightly smaller fragments in the group (see below). Thus, the majority of the [γ-^{32}P]ATP label appeared to be localized in a region common to all three strains.

Interestingly, we found a very similar pattern of fragments for methionine- and ATP-labeled proteins by using proteases having quite different specificities, such as chymotrypsin, thermolysin, and pronase. This suggested that a particularly protease-sensitive region was exposed preferentially even under the SDS denaturing conditions used in these studies. Similar results were obtained with strains P100 and P85 (data not shown).

To document the origin of these specific fragments further, we oriented them by using antisera specific for certain subregions of the *gag* gene (specifically, the p15 and p12 proteins) and antiserum specific for the *abl* sequences (Fig. 2). Protein P120 was labeled by the in vitro autophosphorylation reaction with [γ-^{32}P]ATP and then subjected to control conditions (Fig. 2, lanes A) or to digestion with increasing amounts of *S. aureus* V8 protease (lanes B through D).

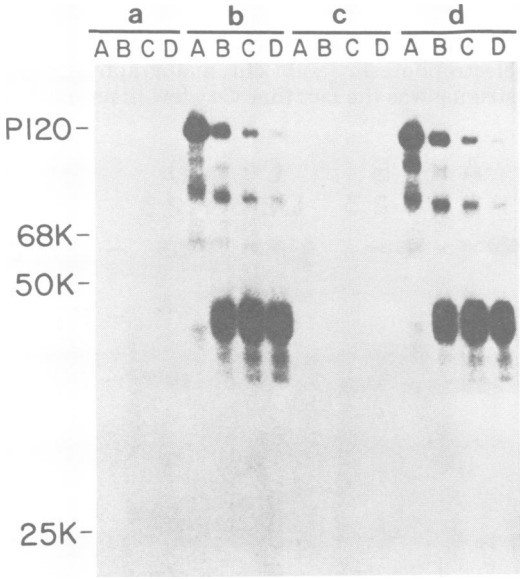

FIG. 2. *Localization of the in vitro kinase phosphate acceptor sites near the amino terminus. Preparations of strain P120 protein labeled with [γ-^{32}P] ATP by the tyrosine auto-phosphorylation reaction were prepared as described in the legend to Fig. 1. Samples were reacted for 30 min in control buffer (lanes A) or with S. aureus V8 protease at 1 μg/ml (lanes B), 3 μg/ml (lanes C), or 10 μg/ml (lanes D); subsequently, these preparations were heat inactivated at 70°C for 5 min and diluted into immunoprecipitation buffer. Portions were re-immunoprecipitated and analyzed by gel electrophoresis. (a) Normal rabbit serum. (b) Rabbit anti-p15 serum. (c) Normal mouse serum. (d) Mouse anti-AbT absorbed to react specifically with the abl sequences of P120 only (30). 68K, 68,000 daltons.*

The cleavage reactions were stopped by heating, and then portions of the preparations were diluted and immunoprecipitated as described above with normal rabbit serum (Fig. 2a), rabbit anti-p15 (the most amino-terminal portion of the *gag* gene and P120) (Fig. 2b), normal mouse serum (Fig. 2c), and mouse anti-AbT (specific for the *abl*-derived portion of P120) (Fig. 2d). Even after heating in SDS, enough antigenic structure was retained so that labeled protein P120 and protein fragments could be recognized by the specific antibodies. The 45,000-dalton set of fragments labeled by the auto-phosphorylation reaction appeared to represent a common amino-terminal region which contained the *gag* sequence and a portion of the *abl* sequences. Similar studies with anti-p12 antiserum gave the same results (see below). Additional control immunoprecipitations with anti-p10 antiserum (p10 was a portion of the *gag* gene not found in A-MuLV proteins) were negative (data not shown). Such immunoprecipitation studies could not prove that the precise amino terminus was present in the 45,000-dalton fragment since small cleavages near the end could have left enough of the p15 sequence intact to be recognized for immunoprecipitation. Some indirect support for the presence of the precise amino terminus in this fragment came from the fact that protein P120 labeled with [^{3}H]acetate gave rise to a labeled 45,000-dalton fragment (but not the 75,000-dalton fragment) after cleavage with *S. aureus* V8 (data not shown). The amino terminus of the *gag* gene protein p15 is known to be blocked by acetylation (14) and should mark this end of the protein P120 molecule as well. Conversion of the label to forms other than the expected acetate group could not be ruled out.

Figure 3 shows the orientations of the carboxyl-terminal fragments of 45,000 daltons for strain P90, 75,000 daltons for strain P120, and 115,000 daltons for strain P160. [^{35}S]methionine-labeled proteins were subjected to control conditions (Fig. 3a) or to cleavage with *S. aureus* V8 protease (Fig. 3b), and the total products were analyzed by gel electrophoresis. Portions of control digestions (Fig. 3c) and *S. aureus* V8-cleaved preparations (Fig. 3d) were diluted and immunoprecipitated with anti-AbT. The carboxyl-terminal fragments of 45,000 daltons (lanes A), 75,000 daltons (lanes B), and 115,000 daltons (lanes C) were recovered with anti-AbT (Fig. 3d). An additional fragment of about 60,000 daltons was found only in digests of strain P160 (Fig. 3d, lane C) and was precipitated with anti-AbT. The precise location of this fragment within the *abl* region was not identified further.

Control sera, including normal mouse serum, normal rabbit serum, and rabbit anti-*gag* gene

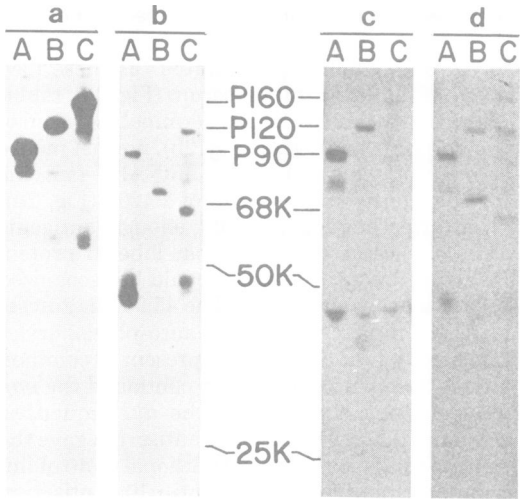

FIG. 3. *Variation in the abl-specific regions of different A-MuLV strains. [^{35}S]methionine-labeled strain P90 (lanes A), P120 (lanes B), and P160 (lanes C) proteins were prepared and isolated by immunoprecipitation as described in the legend to Fig. 1. Samples from control preparations (a) or V8-digested preparations (10 μg/ml; 30 min; 37°C) (b) were analyzed on a 10% polyacrylamide gel without further processing. Samples from control preparations (c) and digested preparations (d) were diluted and re-immunoprecipitated with anti-AbT rendered specific for abl sequences by absorption. 68K, 68,000 daltons.*

product serum, did not precipitate any of these carboxyl-terminal fragments (data not shown).

In vitro and in vivo phosphorylation sites. To begin a study of phosphorylation sites (Fig. 4), we compared protein P120 preparations labeled by in vitro auto-phosphorylation (Fig. 4A and B) and labeled in vivo with $^{32}P_i$ (Fig. 4C and D). Each preparation was subjected to control incubation (lanes 1) or to digestion with increasing amounts of *S. aureus* V8 protease (lanes 2 and 3) and then diluted and immunoprecipitated with anti-p12 serum (Fig. 4A and C) or anti-AbT (Fig. 4B and D). Most striking were the similar patterns obtained with the two labeled preparations. The predominant fragments in both in vitro- and in vivo-labeled preparations included the amino-terminal 45,000-dalton bands which reacted with anti-p12 serum and anti-AbT and a series of smaller fragments (about 15,000 to 20,000 daltons), which were reactive only with anti-p12 serum (Fig. 4A, lane 3, and Fig. 4C, lane 3). These small fragments were not precipitable with anti-p15 serum (data not shown). A small amount of the 75,000-dalton fragment from the carboxyl-terminal (*abl*) region was recovered with anti-AbT from the in vivo-labeled preparation only (Fig. 4D, lane 2). The 75,000-dalton fragment was not recovered from any of the in vitro-labeled preparations. This 75,000-dalton fragment was more sensitive to further proteolysis.

The *abl*-specific carboxyl-terminal fragments (75,000-dalton fragment from protein P120 and 115,000- and 60,000-dalton fragments from protein P160) could be recovered in higher yields by using a lower ratio of protease to substrate or by following the kinetics of digestion (Fig. 5). This was true when $^{32}P_i$-labeled P120 (Fig. 5A) or P160 (Fig. 5B) was digested under control conditions (lanes 1) or with 1, 2, or 4 μg of *S. aureus* V8 protease per ml (lanes 2 through 4, respectively) for 20 min.

Although the partial protease digests showed extensive apparent homologies between the in vitro and in vivo phosphate-labeled preparations of protein P120, a detailed comparison by two-dimensional peptide mapping gave a very different picture (Fig. 6). Protein P120 labeled in vivo (panel A) with $^{32}P_i$ or in vitro with [γ-^{32}P]ATP (panel B) was limit digested with α-chymotrypsin, and the peptides were analyzed by thin-layer electrophoresis and chromatography. Most striking was the fact that very few (if any) of the

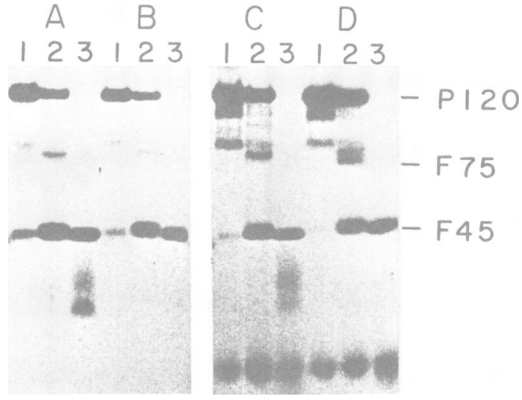

FIG. 4. *Partial protease maps of in vitro- and in vivo-phosphorylated P120. Strain P120 protein isolated by immunoprecipitation and labeled by the in vitro auto-phosphorylation reaction (A and B) was prepared as described in the legend to Fig. 1. In vivo-phosphorylated P120 was isolated by immunoprecipitation from cells labeled with $^{32}P_i$ (C and D) (2 mCi/5 × 10^6 cells; 4 h at 37°C in phosphate-free medium containing 10% dialyzed calf serum). Samples from control reactions (lanes 1) or from 3-μg/ml digests (lanes 2) or 10-μg/ml digests (lanes 3) were heat inactivated, re-immunoprecipitated with goat anti-p12 (A and C) or mouse anti-AbT specific for abl sequences (B and D), and analyzed as described above. The positions of the important 75,000-dalton (F75) and 45,000-dalton (F45) cleavage fragments are also shown.*

peptides of the in vivo-labeled material migrated precisely the same as the in vitro-labeled peptides. The in vitro-labeled peptides included at least 10 to 12 distinct species, all of which were labeled on tyrosine residues (28). There were at least 10 in vivo-labeled peptides (many did not resolve well in ascending chromatography); some of these were labeled on serine, and some were labeled on tyrosine (25, 28; unpublished data). Very similar observations were made by Reynolds et al. (16) and Sefton et al. (25), who also noted the lack of common sites for in vivo- and in vitro-labeled P120. Thus, although the regional localization was similar for a portion of the phosphates on in vitro- and in vivo-labeled P120, the specific sites were distinct. Two-dimensional peptide maps of in vitro-labeled protein P160 and P90 preparations were indistinguishable from the map of protein P120 (data not shown). However, when protein P92 (the kinase-negative protein) was labeled in a *trans* assay by co-immunoprecipitation with protein P120 (33) before the in vitro kinase reaction was carried out, the pattern was different. The peptides labeled in protein P92 represented a subset of those labeled in protein P120. The sites reactive in the *trans* kinase assay represented only a portion of the sites labeled in the auto-phosphorylation reaction (data not shown).

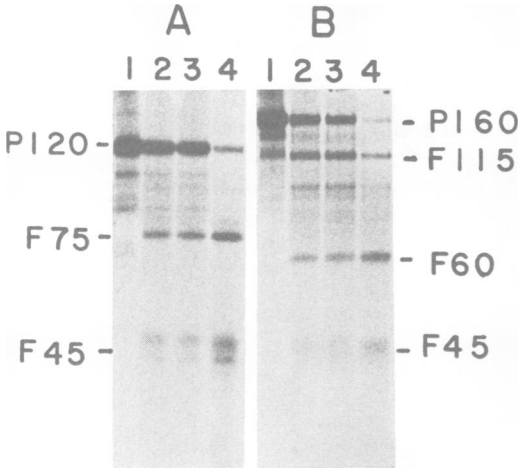

FIG. 5. *Recovery of abl-specific peptide fragments. $^{32}P_i$-labeled strain P120 (A) and P160 (B) proteins isolated by immunoprecipitation were subjected to control conditions (lanes 1) or were digested for 20 min at 37°C with S. aureus V8 protease at 1 μg/ml (lanes 2), 2 μg/ml (lanes 3), or 4 μg/ml (lanes 4). Samples were analyzed on SDS-10% polyacrylamide gels developed by autoradiography. F115, Fragment having a molecular weight of 115,000.*

Mutant strains lose specific in vivo phosphorylation sites. Although the in vitro kinase-labeled two-dimensional peptide maps of the different A-MuLV strains were very similar, the in vivo phosphate-labeled maps were distinct (Fig. 7). Only some spots well separated from the base line were numbered. Strain P120 contained four distinct peptides (peptides 1 through 4). Strain P90, which lacked the carboxyl-terminal 30,000 daltons of strain P120, lacked peptide 2. Strain P92, which had an internal deletion in the *abl* sequences of strain P120, lacked peptide 4 but retained peptides 1, 2, and 3. Strain

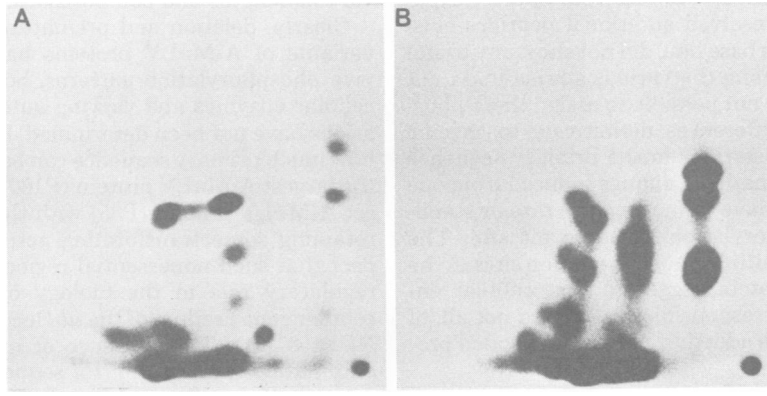

FIG. 6. *Two-dimensional peptide maps of in vitro- and in vivo-phosphorylated P120. Strain P120 protein labeled by the in vitro auto-phosphorylation reaction (B) or labeled in vivo with $^{32}P_i$ (A) was isolated by immunoprecipitation and gel electrophoresis. Labeled peptide was limit digested from gel slices with α-chymotrypsin, concentrated by lyophilization, and then analyzed on cellulose thin-layer plates. Electrophoresis was from right to left at 1 kV for 1 h in formic acid-acetic acid-water (5:15:80). Plates were dried and chromatographed in an ascending buffer containing pyridine, butanol, acetic acid, and water (25:32.5:5:20) from bottom to top. Plates were autoradiographed with intensifying screens to shorten exposure times.*

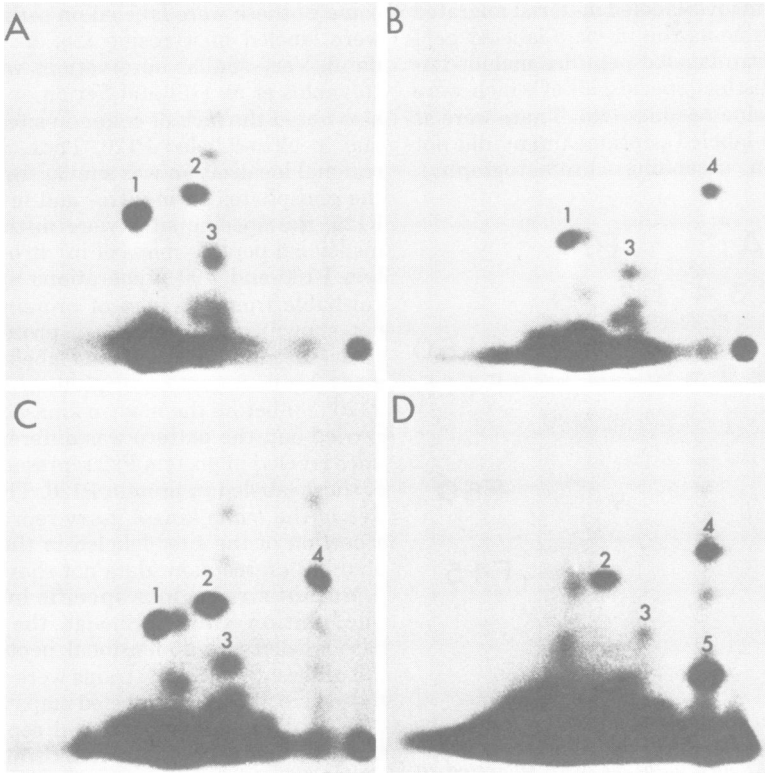

FIG. 7. *A-MuLV strain variants lose specific in vivo phosphorylation sites.* $^{32}P_i$ *in vivo-labeled strain P92 (A), P90 (B), P120 (C), and P160 (D) proteins were isolated by immunoprecipitation, digested, and analyzed as described in the legend to Fig. 6.*

P160, which contained an additional sequence in the *abl* region, lacked peptide 1, but a new peptide (peptide 5) was resolved. Shorter exposures, which resolved additional peptides clustered along the base line, did not show any useful differences among the various strains (data not shown). It was not possible to assign these phosphorylation differences definitively to specific deletions or insertions in the primary sequence since a conformational change induced from one change could have influenced the rate or stability of phosphorylation at a distant site. The presence of multiple phosphorylation sites in the strain P92 protein suggested that cellular enzymes may be responsible for most, if not all, of the phosphates added to A-MuLV-encoded proteins in vivo.

DISCUSSION

The results of one- and two-dimensional peptide mapping studies of the various A-MuLV proteins are in good agreement with the structures predicted by nucleic acid analyses (7, 8). The A-MuLV proteins contained a common amino-terminal segment of about 40,000 to 45,000 daltons and carboxyl-terminal regions of varying lengths and compositions.

Clearly, deletion and premature termination variants of A-MuLV proteins have altered in vivo phosphorylation patterns, but the roles of cellular enzymes and varying auto-phosphorylations have not been determined. It is surprising how much primary sequence can be deleted from the largest A-MuLV protein (P160) to the smallest A-MuLV protein (P85) with the protein still retaining some transforming activity. We suspect that such nonessential regions may play a regulatory role in the biology of the normal cellular gene product of the *abl* locus, designated NCP150 (31). The presence of multiple phosphorylation sites in the *abl* sequences and the deletion of specific sites in well-studied deletion and termination mutants may be useful in elucidating such control regions.

The transformation-defective, in vitro kinase-negative mutant P92 lacked only a single major phosphorylation site in vivo (Fig. 7, peptide 4). We suspect that cellular enzymes are responsible for the majority of the phosphorylations in vivo,

although it is possible that strain P92 may carry out some auto-phosphorylation in vivo. The number and possible functions of the cellular kinases that recognize the *abl*-specific sequences remain unknown.

The in vitro tyrosine-specific auto-phosphorylation activity labels within the amino-terminal region from 40,000 to 45,000 daltons, and a number of sites can be utilized. It is not known whether all of these sites fall within the *gag* region. More extensive digestions of the 45,000-dalton amino-terminal piece do generate smaller labeled fragments that immunoprecipitate with anti-*gag* protein sera but not with anti-AbT (Fig. 4). This suggests that some of these sites are within the *gag* region, but we cannot rule out the possibility of some labeling in the *abl* sequences. The specific peptides labeled bear little resemblance to the peptides in in vivo phosphate-labeled preparations (Fig. 6). It seems likely that the in vitro kinase activity measured in immunoprecipitates or on partially purified protein P120 (28) represents an activity important in vivo but not the precise reaction found within cells. Deciphering the role of the A-MuLV kinase activity in vivo has been approached by several groups. Hunter (10) and Sefton and co-workers (25) have demonstrated an elevated phosphotyrosine level in A-MuLV-transformed cells. This global effect is correlated with A-MuLV transformation, but defining which cellular substrates are specifically phosphorylated by the A-MuLV kinase will require further study. Recently, Dasgupta et al. (A. Dasgupta, D. Baltimore, M. Spector, R. Pepinski, V. Vogt, and E. Racker, manuscript in preparation) have shown that partially purified preparations of A-MuLV P120 can specifically phosphorylate in vitro a purified regulatory protein (designated PKS) involved in the cascade-like control of cell membrane ATPase activity (M. Spector, S. O'Neal, and E. Racker, J. Biol. Chem., in press). Whether this specific reaction can account for the function of the A-MuLV transforming protein in vivo is not known, but it provides a model system for further analysis.

Although the roles of specific in vivo phosphorylation sites on the A-MuLV protein itself are not known, the possibility that cellular protein kinases may modulate A-MuLV kinase activity needs further investigation (12, 22).

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LITERATURE CITED

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