JOURNAL OF VIROLOGY, Dec. 1979, p. 1041-1045
0022-538X/79/12-1041/05$02.00/0

Vol. 32, No. 3

The Nonstructural Component of the Abelson Murine Leukemia Virus Polyprotein P120 Is Encoded by Newly Acquired Genetic Sequences

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Received for publication 26 June 1979

The Abelson leukemia virus (AbLV) polyprotein P120 is compared to translational products representing the entire Moloney murine leukemia virus (MuLV) genome on the basis of [^{35}S]methionine tryptic peptide composition. Three methionine-containing tryptic peptides present in Moloney Pr65^{gag} are each shown to be present in both Pr75^{gag} and in Pr180$^{gag\text{-}pol}$. Of these, one peptide, corresponding to Moloney MuLV p12, but neither of two p30-specific peptides are present in AbLV P120. Among the 12 remaining methionine-containing peptides present in AbLV P120, many, if not all, are unique to AbLV P120 and not shared by either Moloney MuLV Pr180$^{gag\text{-}pol}$ or Pr82^{env}.

Abelson murine leukemia virus (AbLV) was originally isolated subsequent to passage of Moloney murine leukemia virus (MuLV) in a BALB/c mouse (1). In the presence of appropriate helper virus, AbLV transforms both lymphoid cells (10) and embryo fibroblasts (12) in cell culture and induces a rapid B-cell lymphoma in vivo (1). The only known translational product of the replication-defective AbLV genome is a polyprotein of around 120,000 daltons designated AbLV P120 (8, 9, 13). This protein contains antigenic determinants in common with Moloney MuLV p15, p12, and a limited region of p30, but lacks detectable reactivity in immunological assays for Moloney MuLV p10, reverse transcriptase, or envelope glycoprotein, gp70 (8, 13). The nonstructural portion of AbLV P120 is highly phosphorylated (13; W. J. M. Van de Ven, F. H. Reynolds, Jr., and J. R. Stephenson, Virology, in press) and has an associated protein kinase activity (Van de Ven et al., Virology, in press). Based on analogy to the well-characterized avian sarcoma virus-coded transforming protein, pp60^{src} (3, 6), the possibility has been raised that AbLV P120 might possess a transforming function (J. R. Stephenson, A. S. Khan, W. J. M. Van de Ven, and F. H. Reynolds, Jr., J. Natl. Cancer Inst., in press).

Studies to date have not excluded the possibility that AbLV P120 might have resulted from a deletion of Moloney MuLV Pr180$^{gag\text{-}pol}$. Such a deletion could encompass sequences encoding most of p30, p10, and a sufficiently extensive portion of the viral reverse transcriptase to preclude its detection by competition immunoassay. To explore this possibility, we have compared Moloney MuLV and AbLV encoded proteins by [^{35}S]methionine tryptic peptide analysis. The results obtained strongly support the possibility that the nonstructural portion of AbLV P120 is encoded by acquired AbLV-specific genetic sequences not represented in the Moloney MuLV helper virus genome.

Cells were grown in Dulbecco's modification of Eagle medium supplemented with 10% calf serum (Colorado Serum Co., Denver). Cell lines included NIH/3T3 cells productively infected with Moloney MuLV and a subclone of a fetal mink lung cell line, CCL64 (American Type Culture Collection, Rockville, Md.), nonproductively transformed by AbLV, designated 64Ab-2 (11). Exponentially growing cultures were labeled with [^{35}S]methionine, and, after detergent disruption of the cells, virus-specific polypeptides were immunoprecipitated with monospecific antisera (9). Viral proteins were further purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5), visualized by autoradiography and appropriate bands cut from the dried gels. Gel slices were washed in 10% methanol, lyophilized, and subjected to tolylsulfonyl phenylalanyl chloromethyl ketone-trypsin, (Worthington Biochemicals, Freehold, N.Y.) digestion (Van de Ven et al., Virology, in press). After trypsinization, digests were incubated for 2 h at 0°C in 0.1 ml of

chilled performic acid (30% H_2O_2 and 90% formic acid [1:9] preincubated for 2 h at room temperature). Samples were subsequently diluted with 2 ml of H_2O, frozen, and lyophilized. Detection of radiolabeled peptides by fluorography was enhanced by spraying cellulose-coated thin-layer chromatography glass plates (EM Laboratories, Inc., Elmsford, N.Y.) with ether containing 7% diphenyloxazole (2).

For isolation of AbLV and Moloney MuLV translation products for tryptic peptide analysis, AbLV nonproductively transformed mink cells, and NIH/3T3 cells infected with Moloney MuLV were labeled at high specific activity with [^{35}S]methionine. Virus-specific proteins, including Moloney MuLV Pr180$^{gag\text{-}pol}$, Pr75^{gag}, Pr65^{gag}, Pr82^{env}, one Moloney MuLV structural protein, p30, and AbLV P120, were immunoprecipitated by appropriate monospecific sera and subjected to SDS-PAGE. Anti-Rauscher MuLV gp70 was used for precipitation of Pr82^{env}, anti-Rauscher MuLV p30 was used for precipitation of Pr180$^{gag\text{-}pol}$, Pr75^{gag}, Pr65^{gag}, and p30, and anti-Rauscher MuLV p12 was used for precipitation of AbLV P120. The specificity of these sera have previously been defined (9). With the exception of Moloney MuLV Pr180$^{gag\text{-}pol}$, which appeared as a doublet, each of the proteins migrated as single, well-defined bands at their expected molecular weights (Fig. 1).

The results of tryptic peptide analysis of the Moloney MuLV translational products are summarized in Fig. 2 and 3. The most complex pattern was that exhibited by Moloney MuLV Pr180$^{gag\text{-}pol}$. A total of 17 major [^{35}S]methionine-labeled peptides were resolved (Fig. 2). A comparison of the tryptic peptide composition of the two high-molecular-weight components of Pr180$^{gag\text{-}pol}$ revealed no significant differences (data not shown). As predicted on the basis of their known methionine contents, Pr65^{gag} (Fig. 3A) and Pr75^{gag} (Fig. 3B) both contained three major methionine labeled tryptic peptides, two of which were p30 specific (Fig. 3C). Each of these three major peptides, designated 1, 2, and 3, are represented in Pr180$^{gag\text{-}pol}$, consistent with the contention that Pr180$^{gag\text{-}pol}$ represents a read-through translational product of the *gag-pol* region of the Moloney MuLV genome (4, 7). Tryptic analysis of Moloney MuLV Pr82^{env} revealed three major [^{35}S]methionine-labeled peptides, none of which were present in Pr180$^{gag\text{-}pol}$.

Tryptic peptide analysis of [^{35}S]methionine-labeled AbLV P120 revealed 12 major and several minor tryptic peptides (Fig. 4). Of these, one major peptide (no. 1) corresponded to a peptide common to Pr65^{gag}, Pr75^{gag}, and Pr180$^{gag\text{-}pol}$,

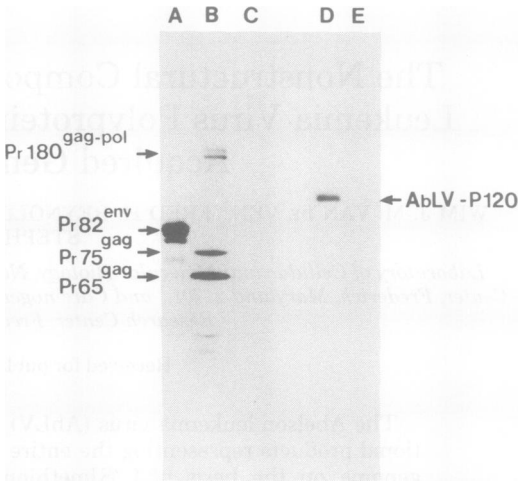

FIG. 1. *Immunoprecipitation and SDS-PAGE analysis of [^{35}S]methionine-labeled viral proteins. NIH/3T3 cells productively infected with Moloney MuLV (A to C) and mink cells nonproductively transformed by AbLV (D and E), were labeled for 15 min in [^{35}S]methionine (150 μCi/ml, specific activity, 1,075 Ci/mmol, Amersham Corp., Arlington Heights, Ill.)-containing medium as described previously (13) and lysed in phosphate-buffered saline containing 1.0% Triton X-100, 0.5% deoxycholate, 0.1% SDS, and 0.1% unlabeled methionine (PBSTDSM). After clarification of lysates at 50,000 rpm for 1 h, viral-specific proteins were complexed with monospecific goat anti-Rauscher MuLV gp70 (A), anti-Rauscher MuLV p30 (B), and anti-Rauscher MuLV p12 (D). In control experiments (C and E), normal goat serum was used. Immune complexes were adsorbed with protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, N.J.), washed three times in PBSTDSM, and analyzed by SDS-PAGE analysis by the method of Laemmli (5) using a 5 to 20% polyacrylamide gradient slab gel. Radiolabeled proteins were visualized by autoradiography.*

while two other peptides (no. 2 and 3) also present in each of the Moloney MuLV precursor polyproteins were missing. These findings are consistent with the finding that peptide no. 1 is p12 specific (data not shown), while peptides no. 2 and 3 are p30 derived (Fig. 3C), and thus establish that those regions of p30 which correspond to the two methionine residues are not represented in AbLV P120. Although we cannot exclude the possibility that some of the 11 remaining major AbLV P120 methionine-labeled tryptic peptides might correspond to peptides contained within Moloney MuLV Pr180$^{gag\text{-}pol}$, certainly the majority, if not all, of these are AbLV P120 specific. Of the three Moloney MuLV Pr82^{env} peptides resolved, none was present in AbLV P120.

These results, summarized diagramatically in

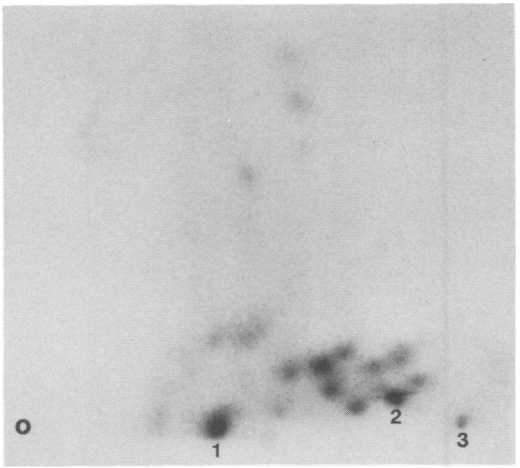

FIG. 2. *Two-dimensional tryptic peptide map of [^{35}S]methionine-labeled Pr180$^{gag\text{-}pol}$. [^{35}S]methionine-labeled Pr180$^{gag\text{-}pol}$ was purified by immunoprecipitation and SDS-PAGE and digested with tolysulfonyl phenylalanyl chloromethyl ketone-trypsin as described in the text. The tryptic digest (6,000 cpm) was resuspended in electrophoresis buffer (acetic acid-formic acid-water, 15:5:80), spotted on a cellulose thin-layer glass plate* (O), *and separated in the first dimension by electrophoresis (left to right) and in the second dimension by ascending chromatography in buffer containing butanol-pyridine-acetic acid-water (35:25:5:20). Tryptic peptides specific to p12 (no. 1) and p30 (no. 2 and 3) are indicated.*

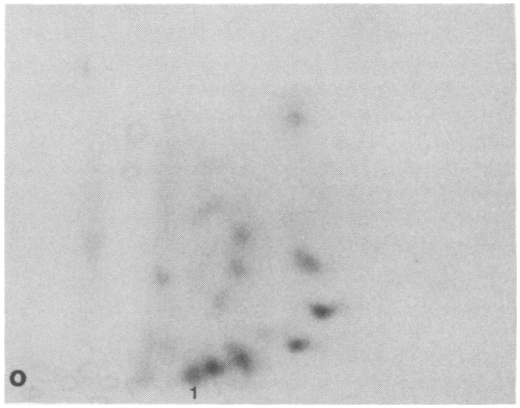

FIG. 4. *Two-dimensional tryptic peptide maps of [^{35}S]methionine-labeled polyproteins encoded by AbLV. Mink cells nonproductively transformed by AbLV were labeled with [^{35}S]methionine, and the radiolabeled polyprotein AbLV P120 was purified and subjected to tryptic peptide analysis as described in the legend to Fig. 2.*

Fig. 5, establish that AbLV P120 contains several [^{35}S]methionine tryptic peptides not represented in Moloney MuLV Pr180$^{gag\text{-}pol}$. This finding almost certainly excludes the possibility that synthesis of AbLV P120 might occur as the result of an inphase deletion within the *gag-pol* region of the Moloney MuLV genome. Thus the results strongly argue that the nonstructural compo-

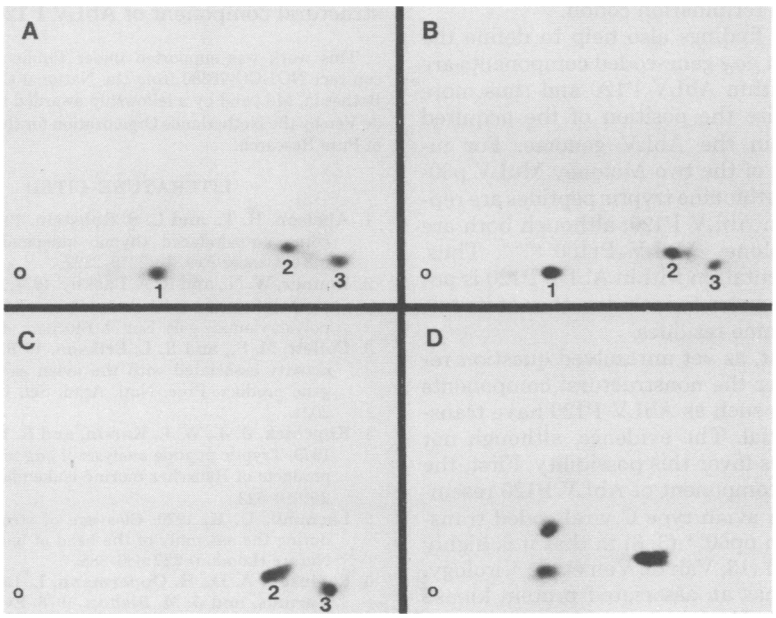

FIG. 3. *Two-dimensional tryptic peptide maps of [^{35}S]methionine-labeled Moloney MuLV gag- and env-gene-coded proteins. [^{35}S]methionine-labeled Moloney MuLV Pr75^{gag} (A), Pr65^{gag} (B), p30 (C), and Pr82^{env} (D) were subjected to tryptic peptide analysis as described in the legend to Fig. 2. Approximately 15,000 cpm of each tryptic digest was applied to the thin-layer plates.*

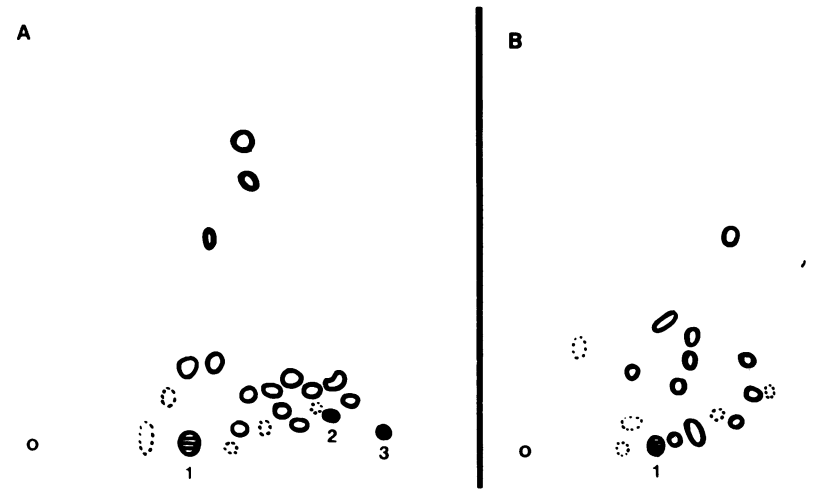

FIG. 5. *Schematic drawing of the [^{35}S]methionine-labeled tryptic peptide maps of Moloney MuLV Pr180$^{gag\text{-}pol}$ (A) and AbLV P120 (B). Tryptic peptides corresponding to those present in p12 (no. 1) and p30 (no. 2 and 3) are designated ⊜ and ●, respectively. Minor tryptic peptides are designated ○.*

nent of AbLV P120 is encoded by cellular genetic sequences acquired in the generation of AbLV genomic RNA. Although the possibility that AbLV P120 is synthesized as the result of a frameshift mutation within the *gag* region of the AbLV genome cannot be excluded, it seems highly unlikely that such an extensive genomic sequence could be translated out of phase before encountering a termination codon.

The present findings also help to define the extent to which *gag* gene-coded components are represented within AbLV P120 and thus more precisely localize the position of the acquired sequence within the AbLV genome. For instance, neither of the two Moloney MuLV p30-specific [^{35}S]methionine tryptic peptides are represented within AbLV P120, although both are present in Moloney MuLV Pr180$^{gag\text{-}pol}$. Thus, the p30 representation within AbLV P120 is not sufficiently extensive to include either of its two known methionine residues.

An important, as yet unresolved question relates to whether the nonstructural components of polyproteins such as AbLV P120 have transforming potential. The evidence, although not conclusive, does favor this possibility. First, the nonstructural component of AbLV P120 resembles the known avian type C viral-coded transforming protein pp60^{src} (3, 6) in that it is highly phosphorylated (13; Van de Ven et al., Virology, in press) and has an associated protein kinase activity (Van de Ven et al., Virology, in press). Secondly, a number of morphological revertants of AbLV-transformed mink cells have been isolated and found to lack detectable AbLV P120 expression (10a). Finally, despite considerable efforts to date, neither we nor others (13) have been able to identify any translational product of the AbLV genome other than P120. Thus, the weight of evidence favors the possibility that the AbLV genome was derived by genetic recombination between Moloney MuLV genomic RNA and host cell specific transforming sequences and that the latter sequences encode the nonstructural component of AbLV P120.

This work was supported under Public Health Service contract NO1-CO-75380 from the National Cancer Institute, Bethesda, Md., and by a fellowship awarded to W. J. M. Van de Ven by the Netherlands Organization for the Advancement of Pure Research.

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