

A transformation-defective mutant of Abelson murine leukemia virus lacks protein kinase activity

(phosphorylation/lymphoid transformation)

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Contributed by David Baltimore, April 21, 1980

ABSTRACT A transformation-defective mutant of Abelson murine leukemia virus (A-MuLV), called A-MuLV-P92*td*, has been isolated. The mutant encodes a serologically identifiable A-MuLV protein of molecular weight 92,000 (P92) but it lacks the ability to transform either fibroblasts or bone marrow lymphoid cells. In contrast to the protein made by transforming strains of A-MuLV, the protein made by A-MuLV-P92*td* does not become phosphorylated during *in vitro* incubation with [γ - 32 P]ATP. If the protein is mixed with proteins from cells transformed by a functional A-MuLV strain, phosphorylation of P92 occurs, showing that its ability to accept phosphate is not altered by the mutation. These parallel changes provide genetic evidence that the A-MuLV protein is a transforming protein and that its associated protein kinase activity (EC 2.7.1.37) is a crucial part of its transforming ability.

Abelson murine leukemia virus (A-MuLV) is a member of the replication-defective rapidly transforming group of retroviruses (1-3). It was derived during passage in mice of the replication-competent Moloney murine leukemia virus (M-MuLV) and can rapidly induce leukemia *in vivo* as well as transform bone marrow cells and some established mouse cell lines *in vitro* (4-7). Many A-MuLV-transformed lymphoid cells have characteristics of pre-B-lymphocytes (8, 9).

The single-stranded RNA genome of the prototype A-MuLV strain is 5.6 kilobases (kb) in length (10). It is composed of 1320 bases at its 5' end and 730 bases at its 3' end derived from the parental M-MuLV strain and a 3600-base central region derived from unique sequences in normal mouse DNA (refs. 10 and 11; unpublished results). A number of A-MuLV strains have been identified (12); each encodes a protein corresponding to a fusion between the amino-terminal region of the M-MuLV *gag* gene product and a polypeptide encoded by the mouse cell-derived sequences (12-14). These proteins have molecular weights ranging from 160,000 to 90,000. The prototype A-MuLV strain encodes a 120,000 molecular weight protein called P120. We use the notation A-MuLV-P120 to denote this strain. P120 is a phosphorylated nonglycosylated protein, of which a portion is exposed on the exterior of the plasma cell membrane of infected cells (15).

When P120 or the other A-MuLV proteins are incubated *in vitro* with [γ - 32 P]ATP, radioactive phosphate is transferred to one or more tyrosine residues in the protein (16). This behavior is similar to the protein kinase activities (EC 2.7.1.37) associated with the Rous sarcoma virus *src* gene product and the middle T (tumor) antigen of polyoma virus (17-19). Because of the close association of tyrosine-phosphorylating activity with transforming proteins and also because the A-MuLV fusion proteins are the only known gene products of the A-MuLV genome (11), we have assumed that these proteins are responsible for transformation by the various A-MuLV strains. No

direct genetic or biochemical data have yet appeared to support this assumption.

The identification of the transforming gene of Rous sarcoma virus, *src*, and its gene product, pp60^{src}, was greatly aided by the isolation of transformation-defective (*td*) mutants (20). Because Rous sarcoma virus contains genes for replicative functions, assays that detected expression of replication genes in the absence of transformation could be used to identify such mutants. In the case of A-MuLV (and other defective transforming retroviruses) there are no linked functional replication genes that can be used in the selection of *td* mutants. As an alternative one can search for cells that express A-MuLV antigens but are not transformed (21). By such screening procedures, we have isolated a spontaneous *td* mutant of A-MuLV. It encodes an antigenically recognizable A-MuLV protein, but the protein lacks the associated protein kinase activity found in proteins from transforming strains of A-MuLV. The coincident loss of kinase activity and transforming potential lends strong support to the hypothesis that the A-MuLV protein is a transforming gene product that may function as a protein kinase.

MATERIALS AND METHODS

Cell Lines and Viruses. The cell lines used and their derivations were: ANN-1 (A-MuLV-P120-transformed nonproducer NIH/3T3 mouse fibroblast); A2 (A-MuLV-P120-transformed nonproducer NIH/3T3 fibroblast that has changed to producing P90 and P92); 2M3 (A-MuLV-P120-transformed nonproducer lymphoid cell from BALB/c bone marrow); and NIH/3T3 uninfected fibroblasts. Cell lines superinfected with M-MuLV are designated as /M (as in 2M3/M). All cells were cultured as described (22, 23). Assays for helper virus plaque-forming units (24), A-MuLV focus-forming units in fibroblasts (5), and A-MuLV lymphoid cell transformation (22, 23) were as described.

Cell Morphology. Cells were seeded (1×10^5 per ml) on sterile glass microscope slide chambers (Lab-Tek Products, Naperville, IL) and allowed to grow for 2 days prior to washing and fixation with 10% (wt/vol) formalin. Wet mounts were photographed at $\times 250$, using the phase-contrast optics on a Leitz microscope, with Tri-X film (Kodak).

Cell Labeling and Immunoprecipitation. Techniques for [35 S]methionine and [3 H]leucine metabolic labeling of cells and extraction, and details of immunoprecipitation, antisera, and electrophoretic analysis have been published (13, 15, 25-27).

In Vitro Kinase Activity. Immunoprecipitates of A-MuLV proteins were tested for kinase activity in a Mn²⁺-activated assay as described (16).

Abbreviations: A-MuLV, Abelson murine leukemia virus; M-MuLV, Moloney murine leukemia virus; kb, kilobase(s); *td*, transformation-defective.

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RESULTS

Isolation of an A-MuLV *td* Mutant. The A-MuLV *td* mutants arose during the study of a particular A-MuLV stock. The stock was prepared from the A2 cell line, an A-MuLV-P120-transformed nonproducer line derived by A. Shields after single cell–single virus infection of NIH/3T3 cells with a stock of A-MuLV from ANN-1/M cells (10). Originally, the A2 cell line expressed the A-MuLV P120 protein as its only MuLV-related product, but on continued passage over 2 years smaller serologically related polypeptides appeared in the cells and ultimately P120 became undetectable. The major A-MuLV-related polypeptide in the cells became one of 90,000–92,000 molecular weight.

A stock of A-MuLV was prepared by superinfection of these late-passage A2 cells with M-MuLV to give the A2/M cell line. NIH/3T3 cells were then infected with the A2/M stock at a multiplicity of infection of about 0.5 focus-forming units per cell and distributed at about 0.3 cell per microwell. Clones were screened morphologically and both transformed and non-transformed clones were selected and tested for synthesis of A-MuLV and M-MuLV proteins by metabolic labeling and immunoprecipitation. Of 20 transformed clones, all expressed a 90,000–92,000 molecular weight A-MuLV protein. Of 18 nontransformed clones, two expressed a 92,000 molecular weight protein. One of these untransformed clones, called Fl-3, which had no evidence of M-MuLV expression, was selected for further study. One nonproducer transformed clone, called Tx-15, was also selected. (Fl refers to the flat morphology of the cells, Tx to transformed morphology.) For reference, these cell lines and viruses derived from them are described in Table 1.

Growth Properties of Fl-3 and Tx-15 Clones. When grown as monolayers, Fl-3 cells remained tightly adherent to the substratum and nonrefractile, even on prolonged growth and refeeding (Fig. 1 *Left*). The final cell density of Fl-3 cells was somewhat (15–20%) higher than that of NIH/3T3 cells. Tx-15 cells (Fig. 1 *Right*) showed the highly refractile and loosely adherent phenotype seen for NIH/3T3 cells transformed by other A-MuLV strains (5). When Tx-15 cells were seeded as single cells in soft agar (200 per 10-cm plate), over 75% grew into macroscopic colonies (1- to 3-mm diameter) within 2–2.5 weeks. Fl-3 cells plated under identical conditions showed no macroscopic colonies after 3 weeks. If the plates were refed weekly, however, and the incubation was continued for 5–6 weeks, small colonies (0.5- to 1-mm diameter) representing about 10% of the initially seeded cells were visible. Uninfected NIH/3T3 cells showed no colonies under such conditions. Four of these Fl-3-derived colonies were picked, and when grown on tissue culture plastic dishes they showed the same growth

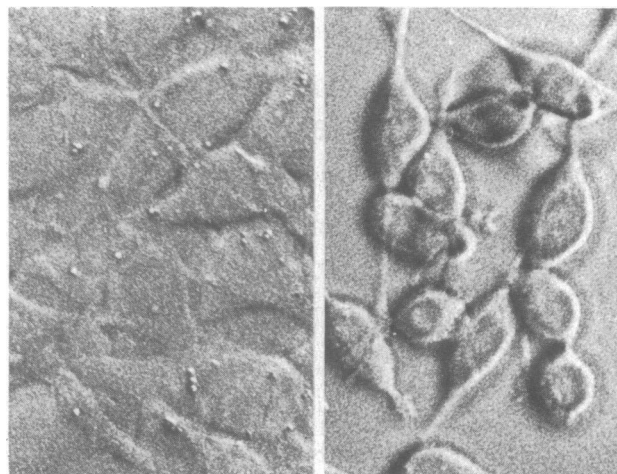


FIG. 1. Cultures of Fl-3 (*Left*) and Tx-15 (*Right*) cells examined by phase-contrast microscopy. ($\times 300$.)

properties as the parent Fl-3 cells. These microcolonies may result from some residual expression of the A-MuLV transforming function.

Serological Comparison of A-MuLV Protein from Tx-15 and Fl-3 Cells. To examine the A-MuLV-related proteins made by Tx-15 and Fl-3 cells, the cells were labeled with [35 S]methionine, and immunoprecipitated products were analyzed by NaDodSO₄/polyacrylamide gel electrophoresis. When a mouse antiserum known to react with the A-MuLV-specific region of the A-MuLV protein (15) was used for immunoprecipitation in the presence of an excess of unlabeled M-MuLV proteins, a 90,000–92,000 molecular weight protein was found in both lines (Fig. 2, lanes B). Normal mouse serum did not precipitate this protein (lanes A). A polyvalent goat anti-M-MuLV serum also precipitated the 90,000–92,000 molecular weight proteins (lanes C) but revealed no helper virus proteins. Sometimes, the 90,000–92,000 molecular weight region from Tx-15 cells was resolved into two separate components, called P90 and P92, but the Fl-3 protein appeared to be a single P92 band.

When these studies were performed, the significance of the P90 and P92 proteins was not apparent. In recent work (unpublished) it has become evident that Fl-3 cells have one A-MuLV provirus but Tx-15 cells have two proviruses. The Fl-3 provirus produces RNA 4.9 kb in length and encodes the P92; the two Tx-15 proviruses make 4.9-kb and 5.6-kb RNAs encoding, respectively, P92 and P90. P90 synthesis is apparently a consequence of a termination mutation in an A-MuLV genome that originally encoded a P120; the P90 is still functional as a transforming protein. P92 synthesis by the 4.9-kb genome is a consequence of a deletion mutation in the A-MuLV-specific region of the A-MuLV genome and this protein is apparently transformation deficient.

Henceforth, the A-MuLV strain in the Fl-3 cells will be referred to as A-MuLV-P92*td*.

P92 Lacks A-MuLV Kinase Activity. We have previously demonstrated that the A-MuLV protein is associated with a protein kinase activity that transfers the γ phosphate of ATP to a tyrosine residue(s) in the A-MuLV protein (16). This reaction appeared to be carried out by the A-MuLV protein itself, but we have not been able to formally rule out the participation of host cell kinase. A functional role for this kinase activity in A-MuLV transformation has not been demonstrated.

To examine the protein kinase activity of the A-MuLV-related P90 and P92 proteins from Fl-3 and Tx-15 cells, the cells were labeled with [3 H]leucine and the A-MuLV proteins were immunoprecipitated. Approximately equal amounts of [3 H]-leucine-labeled 90,000–92,000 molecular weight protein

Table 1. Nomenclature of cells and viruses

Designation	Properties
Cells	
Fl-3	Nontransformed NIH/3T3 cells containing the defective A-MuLV-P92 <i>td</i> provirus
Tx-15	Transformed NIH/3T3 cells containing both the defective A-MuLV-P92 <i>td</i> provirus and a transforming A-MuLV-P90 provirus
Fl-3/M	Fl-3 cells superinfected with M-MuLV
Viruses	
A-MuLV-P90	An A-MuLV strain that makes a protein of molecular weight 90,000
A-MuLV-P92 <i>td</i>	A <i>td</i> strain of A-MuLV that makes a protein of molecular weight 92,000

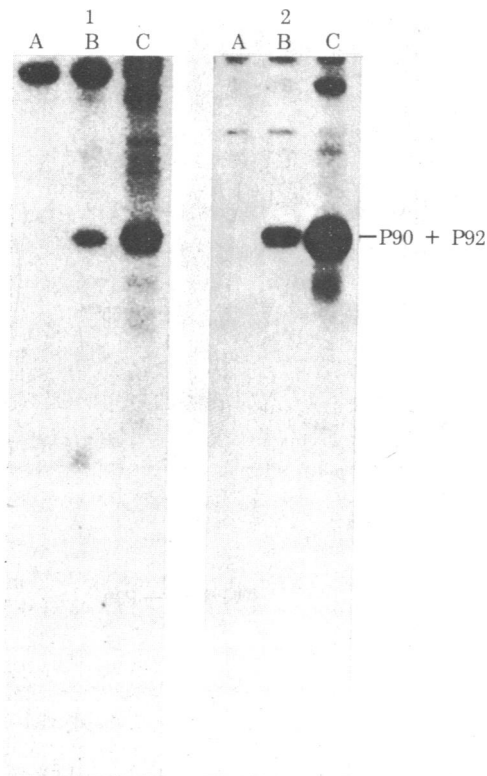


FIG. 2. Serological comparison of P92 and P90 proteins. Cells (2×10^6) labeled with $50 \mu\text{Ci}$ of [^{35}S]methionine (New England Nuclear; $1 \text{ Ci} = 3.7 \times 10^{10}$ becquerels) for 1 hr were extracted, clarified, immunoprecipitated with *Staphylococcus aureus*, and analyzed by NaDodSO₄/10% polyacrylamide gel electrophoresis as described (13, 15, 25, 26). Gel 1 is Fl-3 cells; gel 2 is Tx-15 cells. Lanes A, normal mouse serum. Lanes B, anti-Abelson tumor serum (15) in the presence of unlabeled M-MuLV virion proteins at $100 \mu\text{g}/\text{ml}$. Lanes C, goat anti-M-MuLV serum.

(monitored by fluorography) were recovered from equal numbers of Fl-3 or Tx-15 cells (Fig. 3, lanes A and B). Somewhat smaller apparent breakdown products of P90 and P92 were also present, as seen previously for most A-MuLV proteins (13). When identical immunoprecipitates were assayed for kinase activity by using [γ - ^{32}P]ATP as a substrate and direct autoradiography to monitor ^{32}P incorporation, P92 from Fl-3 cells showed no detectable incorporation above background even on prolonged exposures, whereas the mixture of P90 and P92 from Tx-15 cells was labeled to the same extent as proteins of other transforming strains of A-MuLV (Fig. 3, lanes C and D).

One possible explanation for the lack of ^{32}P incorporation in immunoprecipitates containing P92 alone could be that the protein suffered a mutation that altered the phosphate acceptor site. Previously we had shown that heat-inactivated A-MuLV protein that was unable to label itself could be labeled if mixed and coimmunoprecipitated with an active A-MuLV protein of a distinguishable strain (28). This coprecipitation assay was used to examine the phosphate acceptor site in the P92 protein. Clarified extracts (10^6 cells per ml) were prepared from Fl-3, Tx-15, and an A-MuLV-P160-infected NIH/3T3 cell line. Portions were heat inactivated, A-MuLV proteins were immunoprecipitated from a single lysate or from mixtures, and the precipitates were allowed to react under standard kinase conditions with [γ - ^{32}P]ATP and then were analyzed by NaDodSO₄/polyacrylamide gel electrophoresis. As expected, neither P92 (Fig. 3, lane E) nor heat-treated P92 (lane F) was phosphorylated. If heat-treated Fl-3 extract was mixed with

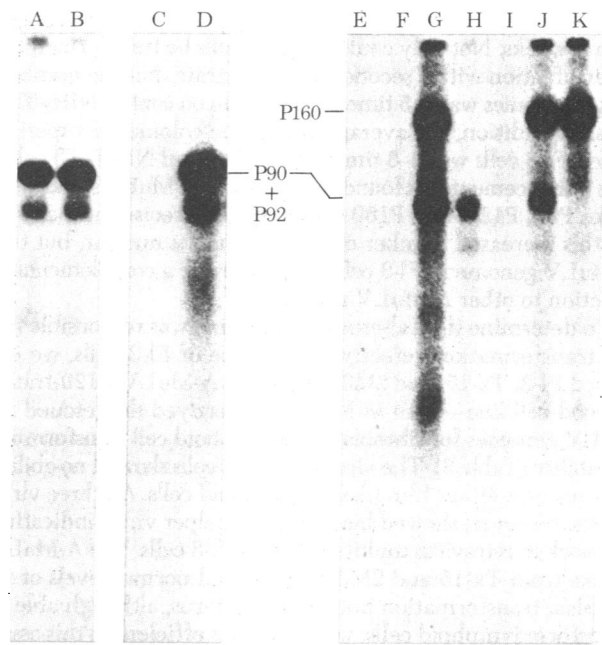


FIG. 3. Labeling of P92 and P90 proteins by incubation of immunoprecipitates with [γ - ^{32}P]ATP. Extracts of [^3H]leucine-labeled cells ($100 \mu\text{Ci}$ per 10^6 cells for 1 hr at 37°C) or unlabeled cells were used alone or in mixtures for kinase reactions as outlined below for measurement of cooperative kinase activity. All kinase reactions were carried out on *S. aureus*-bound immunoprecipitates, formed using goat anti-MuLV serum, in $50 \mu\text{l}$ of 20 mM Tris-HCl, pH 8.0/ 10 mM MnCl₂ containing $1 \mu\text{Ci}$ of [γ - ^{32}P]ATP ($2300 \text{ Ci}/\text{mmol}$, New England Nuclear) at 30°C for 10 min. Proteins were eluted into NaDodSO₄-containing electrophoresis sample buffer and submitted to electrophoresis through NaDodSO₄/10% polyacrylamide gels and developed by fluorography or autoradiography as described (13, 15, 25, 26). Lanes A and B, [^3H]leucine-labeled immunoprecipitates, developed by fluorography, from Fl-3 and Tx-15 cells, respectively. Lanes C and D, kinase reaction products of immunoprecipitated lysates of Fl-3 and Tx-15 cells, respectively, developed by direct autoradiography. The same extracts shown in lanes A and B were used for these reactions. Lanes E-K, kinase activity monitored by autoradiography for: (E) P92 immunoprecipitated from Fl-3 cells; (F) Fl-3 extract heated at 68°C for 1 min prior to immunoprecipitation of P92; (G) heated Fl-3 extract mixed with A-MuLV-P160-infected NIH/3T3 cell extract prior to immunoprecipitation; (H) P90/P92 mixture immunoprecipitated from Tx-15 cells; (I) Tx-15 extract heat-inactivated before immunoprecipitation of P90-P92; (J) heated Tx-15 extract mixed with A-MuLV-P160-infected NIH/3T3 cell extract prior to immunoprecipitation; (K) A-MuLV-P160-infected NIH/3T3 cell extract immunoprecipitated alone.

the P160-containing extract (lane G), then both P160 and P92 were labeled. Thus P92 has a phosphate acceptor site as measured in this coprecipitation assay. A similar set of reactions using Tx-15 extract showed that, in unheated preparations, P90 was labeled with phosphate (lane H), that the activity could be heat-inactivated (lane I), and that heat-inactivated P90 could be labeled when mixed with a p160-containing extract (lane J). The extract with P160 alone labeled only the 160,000 molecular weight protein (lane K). In other experiments (data not shown) P92 was unable to phosphorylate another heat-inactivated A-MuLV protein such as P160 in similar coprecipitation kinase tests.

The Defective Transformation Phenotype of Fl-3 Cells Is a Viral Not a Cellular Defect. To examine whether the lack of transformed phenotype and kinase activity in Fl-3 cells was due to an abnormal cellular rather than viral function, Fl-3 cells and NIH/3T3 cells were infected with wild-type A-MuLV stocks. After 2 hr of exposure to the virus, the cells were removed from the dish with trypsin and suspended in agar at 500

cells per 10-cm plate. Macroscopic agar colonies were counted after 2 weeks. Not only could the Fl-3 cells be transformed by superinfection with a second A-MuLV strain, but the number of agar colonies was 3–5 times greater than on control NIH/3T3 cells. In addition, the average size of the colonies of superinfected Fl-3 cells was 2–3 times that of infected NIH/3T3 cells. This enhancement was found for wild-type A-MuLV stocks that make P90, P120, and P160 proteins. The precise mechanism for this increased number of agar colonies is unclear, but the A-MuLV genome in Fl-3 cells might provide a complementary function to other A-MuLV strains.

To determine if an altered viral genome was responsible for the transformation-defective phenotype of Fl-3 cells, we infected Fl-3, Tx-15, and 2M3—a control A-MuLV-P120-transformed cell line—with M-MuLV and assayed the rescued A-MuLV genomes for fibroblast and lymphoid cell transforming capability (Table 2). The virus from Fl-3 cells showed no ability to transform either fibroblast or lymphoid cells. All three virus stocks, however, showed high levels of helper virus, indicating no block to retrovirus multiplication in Fl-3 cells. The A-MuLV stocks from Tx-15 and 2M3 cells showed normal levels of fibroblast transformation but the Tx-15 virus, although able to transform lymphoid cells, was much less efficient in this assay than the 2M3-derived virus. Reduced lymphoid cell transforming ability by another A-MuLV-P90 strain has been observed (unpublished data).

The lack of transformation by the virus stock rescued from Fl-3 cells could be a consequence of either an inability to rescue any A-MuLV genome from Fl-3 cells or an intrinsic transformation defect in a rescued A-MuLV genome from the cells. To determine if an A-MuLV-P92td genome was rescued from Fl-3 cells and passed to NIH/3T3 cells, we assayed for the presence of the 92,000 molecular weight A-MuLV-specific protein in cells infected by the virus stock rescued from Fl-3 cells. When NIH/3T3 cells were mass infected with an undiluted Fl-3/M stock and allowed to grow for 4 days, no alteration of growth morphology was evident compared to uninfected NIH/3T3 cells. Two days later the cells were labeled for 1 hr with [³⁵S]-methionine, extracted, and clarified for immunoprecipitation. As expected, the cells contained all three of the major helper M-MuLV precursor proteins (29): Pr65^{gag}, Pr80^{env}, and Pr180^{gag-pol} (Fig. 4, lanes C–F). In addition, they contained a 92,000 molecular weight band that contained both A-MuLV-specific determinants—monitored by precipitation with anti-Abelson tumor serum (15) (Fig. 4, lane B)—and *gag* gene determinants (lanes C, E, and F). Thus, in the absence of passage of any transforming ability, we could demonstrate the

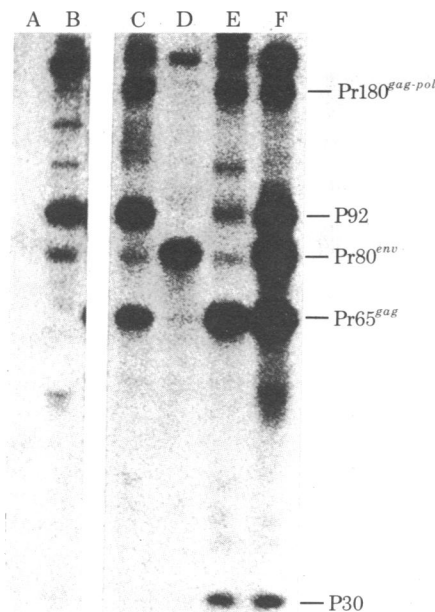


FIG. 4. Transfer of P92 expression by infection. NIH/3T3 cells were mass infected with a stock of A-MuLV from Fl-3/M cells. After 6 days, 5×10^6 cells were labeled with [³⁵S]methionine (200 μ Ci) for 1 hr at 37°C and then extracted, and portions were immunoprecipitated with: Lane A, normal mouse serum; lane B, mouse anti-Abelson tumor serum in the presence of unlabeled M-MuLV proteins at 100 μ g/ml; lane C, rabbit anti-p15/reverse transcriptase; lane D, rabbit anti-gp70; lane E, rabbit anti-p30; lane F, goat anti-M-MuLV virions. Samples were analyzed on a NaDodSO₄/10% polyacrylamide gel developed by fluorography. Lanes A and B are from a 3-day exposure; lanes C–F are from a 1-day exposure.

passage of the A-MuLV-specific gene product of the A-MuLV-P92td strain. Analysis of extracts of this mass-infected population for A-MuLV kinase activity was negative (data not shown). The A-MuLV-P92td genome is thus rescuable but intrinsically defective.

DISCUSSION

By screening nontransformed cells in a population infected with A-MuLV, we have identified a cell line that makes an A-MuLV-related protein but is not transformed. This line contains a virus called A-MuLV-P92td, which can be rescued by superinfection and passed to fresh cells without causing transformation; thus, the virus is truly transformation-defective. Because cells infected with A-MuLV-P92td can form microcolonies in agar after long periods of incubation, the virus may retain a modicum of transforming ability, but by most characteristics it has lost its transforming ability for both fibroblasts and lymphoid cells.

We were able to isolate A-MuLV-P92td because it still expressed a protein with both *gag* and A-MuLV-specific determinants. The mutation that gave rise to A-MuLV-P92td inactivated both its kinase activity and its transforming ability. In the absence of a total genetic analysis of the A-MuLV-P92td genome, we cannot be certain that it has only a single defect, but, by preliminary restriction enzyme analysis of its DNA provirus, it appears to have suffered a single 700-base deletion of the A-MuLV-P120 genome in the A-MuLV-specific region of the genome. On the assumption that this deletion is the only alteration in A-MuLV-P92td compared to A-MuLV-P120, the

Table 2. Biological properties of A-MuLV strains

Virus stock*	Helper titer, PFU/ml†	Fibroblast-transforming activity, FFU/ml‡	Liquid culture bone marrow lymphoid transformation assay for A-MuLV§
Fl-3/M	1×10^7	0	0/10
Tx-15/M	5×10^6	1×10^6	6/10¶
2M3/M	5×10^5	1.2×10^5	9/9

* Stocks prepared by superinfection of Fl-3, Tx-15, and 2M3 (P120-containing, A-MuLV-transformed, nonproducer) cells with M-MuLV. Filtered 18-hr collections were used.

† Helper virus titers, expressed as plaque-forming units/ml, were measured by S+L- assay (24).

‡ Fibroblast focus units were measured on NIH/3T3 cells (5).

§ Mass infection of BALB/c bone marrow in liquid cultures as described (6, 22). Numbers are transformed cultures per cultures assayed.

¶ Transformation noted on day 15.

|| Transformation noted on day 9.

concomitant loss of kinase activity and transforming activity clearly associates these two parameters. Significantly, the P92 protein can still serve as a substrate for the kinase found in A-MuLV-transformed cells. Thus the mutation in A-MuLV-P92*td* has separated the kinase activity associated with the A-MuLV protein from the ability of the protein to act as a substrate for phosphorylation. One interpretation of this dissociation of functions is that separate regions of A-MuLV-transforming proteins are involved in kinase and substrate activity and that at least the kinase activity is required for transformation. We cannot rule out, however, that the A-MuLV protein induces a cellular kinase and that the induction function is defective in A-MuLV-P92*td*.

Similarities exist between the A-MuLV protein and the avian sarcoma virus *src* gene product, although they have different cellular targets for transformation. Both are found in cell membrane fractions (15, 30) and both appear to catalyze protein kinase reactions that result in the formation of phosphotyrosine (16, 19). *src*-containing viruses can transform fibroblasts but not hematopoietic cells. A-MuLV, by contrast, transforms lymphoid cells as well as NIH/3T3 "fibroblasts," but it does not transform primary fibroblasts and its only activity in animals is transformation of hematopoietic cells (mainly lymphoid cells) (7). Proteins of other retroviruses and DNA tumor viruses also have been associated with protein kinase activity (18, 31–35). How these membrane-associated kinase activities are related to the transforming ability of the viruses remains to be determined, but the commonality of properties of these transforming proteins is remarkable.

The number of A-MuLV strains making different sizes of protein is increasing and has required the development of the somewhat cumbersome nomenclature used in this paper. There is still at least one imprecision in the protein designation used here. P90 from Fl-3 cells and Tx-15 cells is presumably the same protein but we do not know if it is the same as a P90 previously described (12). The new P90 should be designated P90A unless an exact correspondence to the previous P90 can be shown. They may be identical, however, because both viruses making these proteins arose from the A-MuLV-P120 strain, although independently; both arose as a consequence of a small change in the A-MuLV-P120 genome, presumably causing the appearance of a new termination codon (unpublished data); and both are relatively deficient for lymphoid as opposed to fibroblast transformation (Table 2; unpublished data).

This work was supported by U.S. Public Health Service Grant CA-24220 (to N.R.), Program Project Grant CA-24530 from the National Cancer Institute (to N.R.), Grant MV-34K from the American Cancer Society (to D.B.), and by U.S. Public Health Service Grant CA-14051 from the National Cancer Institute (Core Grant to S. E. Luria). O.N.W. was a Helen Hay Whitney Postdoctoral Fellow. N.R. is a recipient of a Research Scholar Award from the American Cancer Society (Massachusetts Division). D.B. is an American Cancer Society Research Professor.

1. Graf, T. & Beug, H. (1978) *Biochim. Biophys. Acta* **516**, 269–300.
2. Van Zaane, D. & Bloemers, H. P. J. (1978) *Biochim. Biophys. Acta* **516**, 249–268.

3. Fischinger, P. J. (1980) in *Molecular Biology of RNA Tumor Viruses*, ed. Stephenson, J. R. (Academic, New York), pp. 163–198.
4. Abelson, H. T. & Rabstein, L. S. (1970) *Cancer Res.* **30**, 2208–2212.
5. Scher, C. D. & Siegler, R. (1975) *Nature (London)* **253**, 729–731.
6. Rosenberg, N., Baltimore, D. & Scher, C. D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1932–1936.
7. Rosenberg, N. & Baltimore, D. (1980) in *Viral Oncology*, ed. Klein, G. (Raven, New York) pp. 187–203.
8. Baltimore, D., Rosenberg, N. & Witte, O. H. (1979) *Immunol. Rev.* **48**, 3–22.
9. Siden, E., Baltimore, D., Clark, D. & Rosenberg, N. (1979) *Cell* **18**, 389–396.
10. Shields, A., Goff, S., Paskind, M., Otto, G. & Baltimore, D. (1979) *Cell* **18**, 955–962.
11. Baltimore, D., Shields, A., Otto, G., Goff, S., Besmer, P., Witte, O. & Rosenberg, N. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44**, in press.
12. Rosenberg, N. & Witte, O. N. (1980) *J. Virol.* **33**, 340–348.
13. Witte, O. N., Rosenberg, N., Paskind, M., Shields, A. & Baltimore, D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2488–2492.
14. Reynolds, F. H., Sacks, T. L., Deobaghar, D. N. & Stephenson, J. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3974–3978.
15. Witte, O. N., Rosenberg, N. & Baltimore, D. (1979) *J. Virol.* **31**, 776–784.
16. Witte, O. N., Dasgupta, A. & Baltimore, D. (1980) *Nature (London)* **283**, 826–831.
17. Collett, M. S. & Erikson, R. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2021–2024.
18. Eckhart, W., Hutchinson, M. A. & Hunter, T. (1979) *Cell* **18**, 925–934.
19. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
20. Hanafusa, H. (1977) in *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. R. (Plenum, New York), Vol. 10, pp. 401–483.
21. Sacks, T. L., Hershey, E. J. & Stephenson, J. R. (1979) *Virology* **97**, 231–240.
22. Rosenberg, N. & Baltimore, D. (1978) *J. Exp. Med.* **147**, 1126–1141.
23. Rosenberg, N. & Baltimore, D. (1976) *J. Exp. Med.* **143**, 1453–1463.
24. Bassin, R. H., Tuttle, N. & Fischinger, P. J. (1971) *Nature (London)* **229**, 564–566.
25. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
26. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
27. Witte, O. N. & Baltimore, D. (1978) *J. Virol.* **26**, 750–761.
28. Witte, O. N., Sun, L., Rosenberg, N. & Baltimore, D. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **44**, in press.
29. Eisenman, R. N. & Vogt, V. M. (1978) *B.B.A. Rev. on Cancer* **473**, 187–240.
30. Willingham, M. C., Jay, G. & Pastan, I. (1979) *Cell* **18**, 125–134.
31. Lassam, A. J., Bayley, S. T., Graham, F. T. & Branton, P. E. (1979) *Nature (London)* **277**, 241–243.
32. Tijian, R. & Robbins, A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 610–614.
33. Baumann, E. A. & Hand, R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3688–3692.
34. Griffen, J. D., Spangler, G. & Livingston, D. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2610–2614.
35. Van De Ven, W. J. M., Reynolds, F. H. & Stephenson, J. R. (1980) *Virology* **101**, 185–197.