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Identification of an Abelson murine leukemia virus-encoded protein present in transformed fibroblast and lymphoid cells

(immunoprecipitation/defective genome/*in vitro* translation)

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ABSTRACT **Extracts from lymphoid and fibroblast cell lines transformed by Abelson murine leukemia virus (A-MuLV) contain a protein of molecular weight 120,000 (P120). Immunoprecipitation with specific sera shows that P120 contains regions homologous to the 5'-terminal segment of the MuLV *gag* gene complex—p15, p12, and at least part of p30—but lacks detectable determinants of p10, reverse transcriptase, and the envelope glycoprotein. P120 is phosphorylated and has an intracellular half-life of 3–6 hr. *In vitro* translation of virion RNA from A-MuLV, with Moloney MuLV as helper, yields a product of molecular weight 120,000 with serological reactivity similar to that of the cellular P120. Translation of the RNA from the helper gave no P120. P120 is expressed in all lymphoid and fibroblastic cell lines we have tested that were transformed by A-MuLV but is not detectable in a lymphoid line in which the A-MuLV genome was established by infection but was not responsible for the transformation. Expression of P120 is selectively retained in clones of A-MuLV-transformed lymphocytes that convert to a nonproducer state after loss of expression of helper MuLV intracellular precursors. These results suggest that the P120 product of the A-MuLV genome may be responsible for maintenance of the transformed phenotype of lymphoid and fibroblast cells transformed by the virus.**

Abelson murine leukemia virus (A-MuLV) is a defective leukemia-inducing retrovirus. It was originally isolated from a corticosteroid-treated mouse infected with Moloney murine leukemia virus (M-MuLV) and it generally induces a thymus-independent lymphoma within a short period (3–6 weeks) after *in vivo* inoculation (1). A-MuLV will transform a subpopulation of bone marrow cells *in vitro* (2) and the resulting lines often contain immunoglobulins (3, 4). These and other results suggest that A-MuLV specifically transforms cells of the B lymphocyte lineage (5–7).

A-MuLV is capable of transforming established fibroblastic cell lines. Like the defective murine sarcoma viruses, it requires a helper MuLV to provide essential structural components for its replication and for production of infectious virions but transformed cell lines lacking evidence of replication of the helper virus can be isolated (nonproducer transformed fibroblasts) (8, 9).

Although any replicating MuLV appears to be able to act as a helper for fibroblast transformation, the interaction of A-MuLV with lymphoid target cells is more complex. Recently, Rosenberg and Baltimore (10) and Scher (11) have demonstrated that the successful transformation of lymphoid cells by A-MuLV is dependent on the strain of helper MuLV used. Only strains of MuLV that are themselves highly leukemogenic are efficiently able to provide the helper functions necessary for *in vivo* and *in vitro* transformation of lymphoid cells by A-MuLV. The nature of this interaction is not understood but it appears that the helper plays a role at least for the induction of lymphoid cell transformation.

We have initiated a search for gene products in A-MuLV-transformed cells that could play a role in the transformation process. By immunoprecipitation of labeled cellular extracts we have found a unique protein of molecular weight 120,000 (P120) that bears partial serological homology to certain of the MuLV gene products. The presence of P120 in lymphoid and fibroblast nonproducer cells transformed by A-MuLV, coupled with its cell-free translation from A-MuLV virion RNA, suggests that P120 is a product of the Abelson genome that may be involved in the induction or maintenance of the transformed state.

MATERIALS AND METHODS

Cells and Viruses. Lymphoid cell lines transformed *in vitro* by A-MuLV were isolated from individual bone marrow colonies by using the agarose transformation assay previously described (2). SJL-46 (12), a dimethylbenzanthracene-induced tumor in a thymectomized SJL/J mouse, was a gift of N. Haran-Ghera, Department of Immunology, Weizmann Institute of Science, Rehovot, Israel. L-691-6 is a clone of L-691 cells (13) that were obtained from Paul Arnstein, Viral Carcinogenesis Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD. A-MuLV-transformed mouse fibroblasts were isolated by infecting NIH/3T3 cells at a low multiplicity and cloning after the virus adsorption period. Ab-NRK cells (9) were a gift of E. Scolnick, National Institutes of Health, Bethesda, MD. Methods of cell growth, virus stock preparation, and superinfection of cell lines have been described (2, 10).

***In Vitro* Translation.** Virion RNAs were used to program the mRNA-dependent rabbit reticulocyte lysate system as described (14, 15).

Immune Sera. Rabbit anti-M-MuLV reverse transcriptase, anti-M-MuLV gp70, and anti-M-MuLV p30 have been described (15). Goat anti-Rauscher MuLV p15, p12, and p10 antisera were provided through the Virus Cancer Program of the National Cancer Institute and were prepared by R. Wilsnack, National Cancer Institute. Goat anti-M-MuLV virions have been described (16).

Cell Labeling and Immunoprecipitation. Subconfluent cell monolayers of fibroblast cells or exponential phase suspension cell lines at 2×10^6 cells per ml were labeled for the times indicated in Dulbecco's minimal essential medium containing $\frac{1}{50}$ the normal methionine concentration at 50 μCi of [^{35}S]-methionine (New England Nuclear, Boston, MA) per ml. Cells were labeled with [^{32}P]orthophosphate (50 μCi/ml) (New England Nuclear) in phosphate-free minimal essential medium

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Abbreviations: A-MuLV, Abelson murine leukemia virus; M-MuLV, Moloney murine leukemia virus; FFU, focus-forming units; PFU, plaque-forming units; $NaDodSO_4$, sodium dodecyl sulfate.

for 30 min. Cells were extracted and immunoprecipitated as detailed elsewhere (16). Briefly, cells at 1–2 × 10^6/ml were lysed with 1% Triton X-100/0.5% deoxycholate/0.1% sodium dodecyl sulfate ($NaDodSO_4$)/0.01 M NaH_2PO_4, Na_2HPO_4/0.1 M NaCl, pH 7.5) precleared with normal serum and *Staphylococcus aureus* Cowan strain 1 (17), and then clarified. Immunoprecipitates were made by incubating up to 1 ml of lysate with 5 μl of immune serum and then collecting antigen–antibody complexes with *S. aureus*. ^{14}C-Amino acid-labeled M-MuLV virions were prepared as described (18).

$NaDodSO_4$/Polyacrylamide Gel Electrophoresis. Immunoprecipitates and other samples were denatured in 1% $NaDodSO_4$/1% 2-mercaptoethanol/50 mM Tris·HCl, pH 6.8/10% glycerol and electrophoresed on 20–5% linear polyacrylamide gradient gels as described (19, 20). Gels were fixed and fluorographed as described (21).

Molecular weight estimates were interpolated from known viral proteins of adenovirus type 2 and vesicular stomatitis virus and standard proteins.

RESULTS

Identification of P120 in A-MuLV-Induced Lymphomas. We have screened a number of lymphoma cell lines with a battery of antisera prepared against leukemia virus proteins. To show the specificities of these antisera for virion proteins, e. ıcts of purified virions of M-MuLV labeled with mixed ^{14}C-amino acids were immunoprecipitated at antibody excess and the precipitates were fractionated by $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 1*A*). Lane 1 shows that the anti-reverse transcriptase precipitate contained reverse transcriptase (p85) and the low molecular weight component p15. Note that the migration in $NaDodSO_4$/polyacrylamide gel electrophoresis of p15 is actually close to 9000–10,000 molecular weight. This protein, however, lacks methionine. is not phosphorylated, and can be recovered in the appropriate processing intermediates of the MuLV *gag* gene (O. N. Witte and D. Baltimore, unpublished data), showing that it is homologous to the NH_2-terminal p15 described for Rauscher MuLV (22). The anti-p15 activity of the anti-RT/p15 antiserum could be adsorbed with purified MuLV low molecular weight (10,000–30,000) proteins without affecting the precipitation of the reverse transcriptase (p85) itself, indicating that separate antibody molecules are responsible for the precipitation of the two proteins (data not shown). Anti-gp70 (lane 2), anti-p30 (lane 3), anti-p15 (lane 4), anti-p12 (lane 5), and anti-p10 (lane 6) were reactive predominantly with the antigen used to induce the antiserum. Anti-gp70 precipitated p15E and p12E because they are attached to gp70 under nonreducing conditions (20).

To reveal the proteins encoded by M-MuLV in a virus-induced lymphoma, pulse-labeled ([^{35}S]methionine) cellular extracts from the M-MuLV-induced lymphoma B244 were immunoprecipitated (Fig. 1*B*). The major viral gene products visualized were the polyproteins $Pr65^{gag}$ and $Pr80^{env}$ (23). Some cleavage products, such as the virion structural protein p30, were also evident.

When a lymphoid cell line transformed *in vitro* by A-MuLV plus helper M-MuLV was similarly labeled, extracted, and immunoprecipitated a new component was found. This cell line, called 18-48, was producing both A-MuLV and M-MuLV when it was studied. In spite of this, the labeled extracts contained little of M-MuLV-specific *gag* polyprotein or its cleavage products (Fig. 1*C*). However, the extract had a readily detected protein of 120,000 molecular weight (P120) that was precipitated by the anti-RT/p15 (lane 1) and anti-p30 antisera (lane 3) but not by the anti-gp70 (lane 2). $Pr80^{env}$ was precipitated

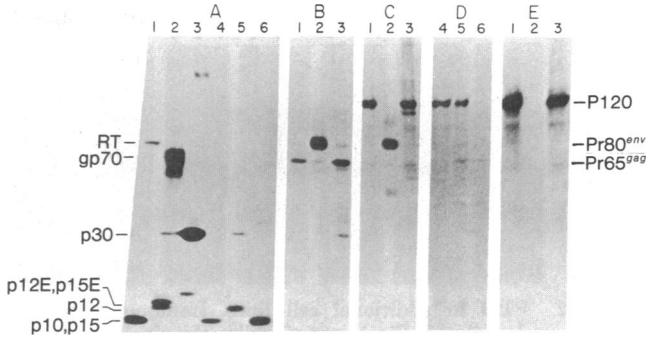

FIG. 1. Identification of P120 in A-MuLV-induced lymphomas. Immunoprecipitates of labeled cell and virus extracts were analyzed on $NaDodSO_4$/polyacrylamide gradient gels by fluorography. (*A*) Specificities of antisera by immunoprecipitation of mixed ^{14}C-amino acid-labeled extracted virion proteins of M-MuLV. Lane 1, rabbit anti-reverse transcriptase (RT)/p15; lane 2, rabbit anti-gp70; lane 3, rabbit anti-p30; lane 4, goat anti-p15; lane 5, goat anti-p12; and lane 6, goat anti-p10. (*B*) [^{35}S]Methionine pulse-labeled (30 min) Moloney MuLV-induced lymphoma B244. Specific sera as above. (*C*) [^{35}S]-Methionine pulse-labeled (30 min) A-MuLV (M-MuLV helper)-induced lymphoma 18-48. Specific sera as above. (*D*) [^{35}S]Methionine pulse-labeled (30 min) and chased (60 min) 18-48 cells immunoprecipitated with *gag* gene antisera anti-p15, p12, and p10. (*E*) [^{32}P]-Orthophosphate pulse-labeled (30 min) 18-48 cells. Specific sera as in (*A*).

by the anti-gp70 antiserum. The reactivity of the anti-RT/p15 antisera was removed completely by absorption with low molecular weight proteins of MuLV (data not shown). A second anti-reverse transcriptase antiserum (from T. Papis) did not precipitate P120 although it did precipitate reverse transcriptase (p85) from virions (data not shown). Thus, P120 appears to contain structural information related to the MuLV *gag* gene products p15 and p30 but lacks detectable homology to the *pol* and *env* gene products. A second component, of molecular weight about 110,000, was precipitated by anti-p30 antiserum (Fig. 1*C*, lane 3). It was not readily precipitated with other anti-p30 antisera or antisera prepared against whole MuLV virions and has not been further analyzed.

Extracts of line 18-48 cells pulse-labeled for 30 min with [^{35}S]methionine and chased for 60 min in complete medium were immunoprecipitated with *gag* gene-specific antisera (Fig. 1*D*). Anti-p15 (lane 4) and anti-p12 (lane 5) were able to precipitate P120 but anti-p10 (lane 6) was unable to do so. In longer pulse-chase experiments, P120 was found to have an intracellular half-life of 3–6 hr. No defined cleavage products have been identified (O. N. Witte and D. Baltimore, unpublished data). Also, line 18–48 cells pulse-labeled with $^{32}PO_4$ were extracted and immunoprecipitated (Fig. 1*E*). P120 was seen to be the major intracellular phosphorylated form precipitable with anti-RT/p15 (lane 1) and anti-p30 (lane 3).

These studies show that P120 is a phosphoprotein that contains homology to the 5′ gene sequence of MuLV (H_2N-p15-p12-p30) but lacks serological homology to the *pol* or *env* gene products or to p10, the COOH-terminal *gag* gene product.

P120 Is Found in A-MuLV Transformed Nonproducer Lymphoid and Fibroblast Lines But May Not Be Required for Replication of the A-MuLV Genome. A-MuLV can transform fibroblast cell lines yielding some nonproducer lines. The A-MuLV component can be rescued from such lines by superinfection with another helper MuLV (8–10). We tested such a transformed nonproducer line, NIH/3T3-derived A2, for the presence of P120 (Fig. 2*A*). Extracts of cells pulse-labeled with [^{35}S]methionine were immunoprecipitated with anti-RT/p15 (lane 1), anti-gp70 (lane 2), or anti-p30 (lane 3). P120 that was precipitable by anti-RT/p15 or anti-p30 was the major viral polypeptide detected. This was the same serological

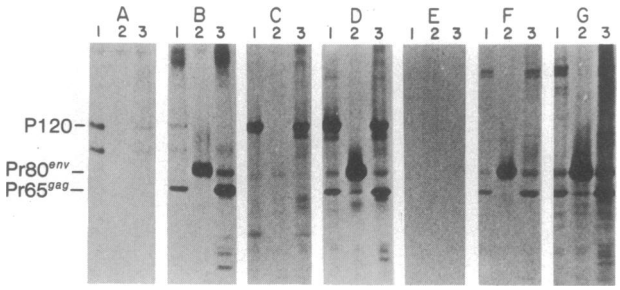

FIG. 2. P120 in additional cell lines. Immunoprecipitation analysis (as described in Fig. 1) was of [^{35}S]methionine pulse-labeled (30 min) cell lines. Lanes were the same as in Fig. 1. (*A*) A-MuLV-transformed NIH/3T3 nonproducer cell line A2. (*B*) M-MuLV-superinfected A2/M cells. (*C*) A-MuLV-transformed lymphoid nonproducer 143-2M3 cells. (*D*) M-MuLV-superinfected 143-2M3/M cells. (*E*) X-ray induced thymic lymphoma L-691-6 cells. (*F*) M-MuLV-superinfected L-691-6/M cells. (*G*) A-MuLV- and M-MuLV-superinfected L-691-6/AM cells.

specificity seen for the P120 of producer lymphoid cells transformed by A-MuLV. A second component of 90,000–100,000 molecular weight was also precipitated by the anti-RT/p15 and anti-p30 antisera. A nonproducer A-MuLV-transformed rat fibroblast line (Ab-NRK obtained from E. Scolnick; ref. 9) contained P120 but not the lower molecular weight component (not shown). Neither fibroblast line had any of the normal M-MuLV precursor proteins.

A2 cells were superinfected with M-MuLV, and a producer clone (A2/M) was derived and tested by immunoprecipitation (Fig. 2*B*). P120 continued to be expressed in these cells. Proteins corresponding to the MuLV precursors Pr65^{gag}, Pr75^{gag}, and Pr80^{env} were also seen (Fig. 2*B*, lanes 1, 2, and 3). Similarly, when a lymphoid nonproducer cell line, 143-2M3 derived from the *in vitro* transformation of bone marrow cells by A-MuLV, was tested, it too contained P120 (Fig. 2*C*). Upon superinfection of 143-2M3 with M-MuLV (Fig. 2*D*), P120 continued to be expressed along with the intracellular M-MuLV precursors Pr65^{gag}, Pr75^{gag}, and Pr80^{env}.

These results suggest that P120 is a product of the A-MuLV genome that is expressed in transformed nonproducer and producing cell lines whenever A-MuLV was the transforming agent. A large number of cell lines have been assayed for P120 to test the generality of the correlation of P120 with A-MuLV-induced transformation (Table 1). Expression of P120 was independent of the type of cell transformed by A-MuLV and the helper virus used in the A-MuLV stock. A-MuLV-transformed fibroblasts of mouse and rat origin and lymphoid cell lines from a number of mouse strains all had the protein. P120 was present in all cases in which A-MuLV induced transformation, regardless of which other MuLV proteins were expressed. When a number of lymphoid cell lines transformed by other agents were examined, none of these cells had detectable P120. Included in this group were several thymic lymphomas induced by the clone of M-MuLV helper used in many A-MuLV stocks and the SJL/46 cell line which resembles most A-MuLV-transformed lymphoid cells with respect to immunologic markers (7).

To test if P120 is required for A-MuLV replication, as opposed to its apparent necessity for A-MuLV transformation, a cell line transformed by a different agent was infected with A-MuLV and a culture producing both A-MuLV and helper M-MuLV was derived. The host cell was L-691-6, a line derived from a thymic lymphoma caused by x-irradiation *in vivo* (13). L691-6 bears the surface antigen and has the biological characteristics of a thymus-derived lymphoma (7, 13). It produces a xenotropic retrovirus but no detectable ecotropic MuLV. We

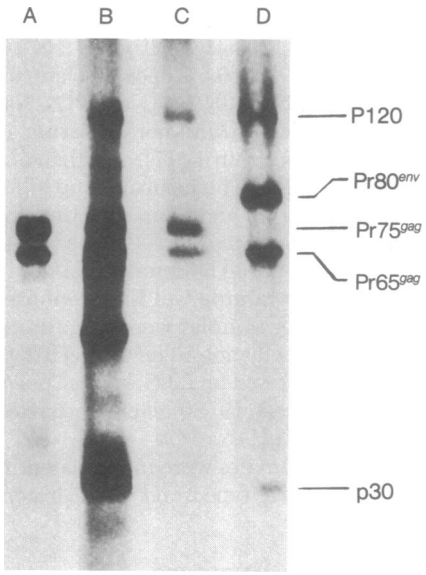

FIG. 3. *In vitro* translation of P120. The mRNA-dependent reticulocyte lysate system (14, 15) was programmed with purified 38S M-MuLV virion RNA subunits or heat-denatured subunits of a mixture of A-MuLV and M-MuLV virion RNA. Samples electrophoresed on a NaDodSO$_4$/10% polyacrylamide gel. (*A*) M-MuLV RNA total translation products labeled with [^{35}S]methionine. (*B*) A-MuLV + M-MuLV RNA total translation product. (*C*) Immunoprecipitation of an aliquot of A-MuLV + M-MuLV product with anti-RT/p15 serum. (*D*) Intracellular standards (P120, Pr65^{gag}, and Pr80^{env}) prepared from 143-2M3/M cells.

superinfected L691-6 with M-MuLV (L691-6/M) or A-MuLV stocks containing M-MuLV as a helper (L691-6/AM). Labeled cell extracts from L691-6 (Fig. 2*E*), L691-6/M (Fig. 2*F*), and L691-6/AM (Fig. 2*G*) were immunoprecipitated with specific sera. None of the cell lines contained P120. This included L691-6/AM which was found to replicate and produce infectious, oncogenic A-MuLV particles (Table 1). L691-6/M and L691-6/AM both contained intracellular precursors of M-MuLV (Pr65^{gag} and Pr80^{env}). Although these results do not eliminate the possibility that very small amounts of P120 may be produced and provide an essential function for A-MuLV replication, reconstruction experiments indicate that L691-6/AM cells express less than 5% of the level of P120 found in lymphoid cell lines (such as line 18-48) transformed by A-MuLV. We therefore conclude that P120 is probably not required for productive A-MuLV infection.

***In Vitro* Translation of P120 from A-MuLV Virion RNA.** To determine if the A-MuLV genome can direct synthesis of P120 in a cell-free system, 60–70S RNA was purified from a mixture of A-MuLV and M-MuLV harvested from a transformed fibroblast cell line, and the RNA was heat denatured to yield 30–38S subunits. As a control, pure 38S MuLV RNA (a kind gift of P. Andersson) was translated. Fractions precipitated from ethanol were used for translation in the mRNA-dependent reticulocyte lysate system (14, 15). Fig. 3*A* shows the pattern of total synthesis for the M-MuLV RNA preparation. No P120 was evident and the previously described viral components corresponding to Pr65^{gag} and Pr75^{gag} were the major translation products (15). When A-MuLV virion RNA was translated (Fig. 3*B*), an additional band of molecular weight 120,000 was detected which comigrated with P120 immunoprecipitated from cellular lysate. Immunoprecipitation with anti-RT/p15 (Fig. 3*C*) showed that the cell-free product had the same serological reactivity as the intracellular P120. P120, Pr80^{env}, and Pr65^{gag} immunoprecipitated from cellular lysates (Fig. 3*D*) are shown as markers. These results show that the A-MuLV

Table 1. Biological phenotypes and intracellular viral proteins of transformed cell lines

Origin	Helper	Cell line	Mouse strain	Ref.	Virus production* PFU/ml	Virus production* FFU/ml	Reverse transcriptase, cpm × 10^{-3}	Proteins $Pr65^{gag}$	Proteins $Pr80^{env}$	Proteins P120
				By A-MuLV						
In vitro transformed bone marrow (lymphoid)	M-MuLV	18-48	BALB/cJ	7	7×10^4	8×10^3	26.6	+	+	+
		18-8	BALB/cJ	7	0	0	12.0	+	+	+
		SWR-4	SWR/J	7	0	0	19.0	−	−	+
		SWR-4/M	SWR/J	—	5×10^4	8×10^4	46.2	+	+	+
		BR 48-1	C57BR/cdJ	10	0	0	0.29	−	+	+
		BR 48-1/M	C57BR/cdJ	10	5×10^5	5×10^4	55.5	+	+	+
		143-2M3	BALB/cAN	10	0	0	0.26	−	−	+
		143-2M3/M	BALB/cAN	10	3×10^5	1×10^5	NT	+	+	+
		TB-7	NIH/Swiss	—	5×10^2	3×10^2	1.0	+	+	+
		TB-6	NIH/Swiss	—	0	0	0.30	−	+	+
		L1-2	C57L/J	7	0	0	0.30	+	+	+
	Friend MuLV	143-3B	BALB/cAN	10	0	0	7.1	−	+	+
	Gross MuLV	131-1	NIH/Swiss	—	0	0	9.6	−	+	+
	Rauscher MuLV	185-1	NIH/Swiss	—	NT	NT	NT	+	+	+
In vitro transformed fibroblast	M-MuLV	A2	(NIH/3T3)†	—	0	0	NT	−	−	+
		A2/M	(NIH/3T3)†	—	3×10^5	1×10^5	42.5	+	+	+
		Ab-NRK	(NRK)†	9	0	0	NT	−	−	+
In vivo lymphoid tumor	M-MuLV	AB4-2	BALB/cJ	—	NT	NT	NT	+	+	+
				By x-ray						
In vivo thymoma	—	L-691-6	C57L/J	10, 13	0	0	0.2	−	−	−
		L-691-6/M	C57L/J	10	7×10^5	NT	NT	+	+	−
		L-691-6/AM	C57L/J	—	7×10^5	2×10^4	28.5	+	+	−
				By carcinogen						
In vivo lymphoma	—	SJL/46	SJL/J	12	<50	0	13.4	+	+	−
				By M-MuLV						
In vivo thymoma	—	MT-4	HaM/ICR CD-1	—	6×10^4	NT	NT	+	+	−
		MT-11	HaM/ICR CD-1	—	0	0	1.1	+	+	−
		B244	BALB/cJ	—	NT	NT	NT	+	+	−

* Virus production was monitored by using infectivity and reverse transcriptase assays according to standard procedures (2, 10). PFU were measured by using the S+L− assay (24); FFU were monitored by using the NIH/3T3 or BALB/3T3 focus assay (8). Reverse transcriptase values represent cpm/10^6 cells. NT, not tested.

† Cell line used for transformation noted, not animal strain.

genome codes for the P120 protein.

Retention of P120 in Cloned Cell Lines That Convert to Nonproducer Status. During the course of these studies the cloned A-MuLV-transformed producer cell line, 18-48, was serially followed for production of M-MuLV [S+L− assay, PFU (24)] and A-MuLV [focus assay, FFU (8)]. Over a 4-month period, both the PFU and FFU titers decreased to almost 1/10 as did the reverse transcriptase activity of released virions.

When line 18-48 cells were subcloned and the clones were tested individually for biological activity, only one in five produced high titers of infectious virus. The intracellular proteins expressed in the four nonproducer subclones (18-48-1, -2, -3, and -5) (<10 FFU/ml) and one producer subclone (18-48-4) (>10^4 FFU/ml) were assayed. Pulse-labeled cellular extracts were immunoprecipitated with an antiserum prepared against disrupted M-MuLV virions that had specificity for *gag* and *env* gene products (Fig. 4). All of the subclones retained the expression of P120 and $Pr80^{env}$. Only the producer subclone retained expression of $Pr65^{gag}$ and $Pr180^{gag\text{-}pol}$ gene products.

DISCUSSION

Of the 16 lymphoid and fibroblastic cell lines transformed by A-MuLV that we have assayed, all contained P120. The occurrence of P120 in nonproducer cells as well as its translation from A-MuLV RNA strongly implies that it is a product of the A-MuLV genome. Although genetic data are required to prove that P120 is involved in A-MuLV-induced cell transformation, it seems likely that P120 has such a role.

P120 is apparently not needed, at least in large amounts, for A-MuLV replication because a producer cell originally transformed by x-rays, L691-6/AM, had no detectable P120. This result is consistent with the provision of all A-MuLV proteins by its helper virus.

The A-MuLV genome has yet to be physically characterized but the recent demonstration of a 28–30S RNA correlating with A-MuLV biological activity suggests that this may be the genome (A. Shields, unpublished data). If so, the genome would be about 5000 bases and could encode a protein as large as 200,000 molecular weight. Thus, P120 might not be the only

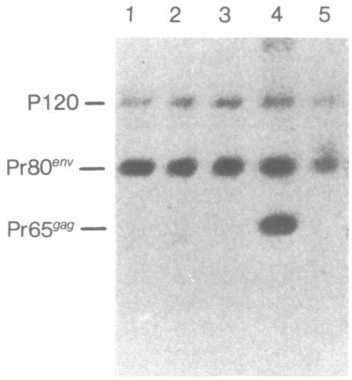

FIG. 4. Maintenance of P120 in the conversion to nonproducer status. [^{35}S]Methionine pulse-labeled (30 min) subclones (1–5) of the A-MuLV-transformed (M-MuLV as helper) producer clone 18-48 were tested for intracellular precursors by immunoprecipitation with a polyvalent goat anti-MuLV virions antiserum. Precipitates were electrophoresed on a $NaDodSO_4$/10% polyacrylamide gel.

A-MuLV-specified product. Retroviruses may have 1000–1500 more bases than are needed to encode their known proteins (25), so it is conceivable that P120 is the only A-MuLV-specified protein. If a protein lacking any M-MuLV-specific antigenicity were synthesized by A-MuLV, we could not have detected it in these studies. In certain cell lines, proteins of molecular weight 100,000–110,000 were seen along with P120 (Fig. 2 *A* and *B*); their sporadic occurrence suggests that they may be degradation products of P120 but they may deserve further attention.

Our serological data imply that the A-MuLV genome encodes some but not all of the *gag* proteins of its parent, M-MuLV. The 5′-terminal portions of the *gag* polyprotein ($Pr65^{gag}$), p15 and p12, are represented in P120, and our M-MuLV-specific anti-p30 recognizes P120 also. Previous studies found p15 and p12 but not p30 determinants among the A-MuLV-specified products (9) and we have also found an anti-p30 antiserum that will not recognize P120. We therefore believe that P120 results from a fusion of the 5′ end of the M-MuLV genome somewhere within the p30 coding region to a genetic region of undetermined origin. Although 30,000–50,000 daltons of P120 are M-MuLV in origin, the rest has no antigenicity that we can yet define. No anti-RT or anti-gp70 antiserum can recognize P120 (except in the case of the strong anti-p15 reactivity of our anti-RT antiserum) and we therefore believe that the A-MuLV genome probably has a genetic region not found in M-MuLV.

Comparison of P120 to the gene products of several other defective leukemia/sarcoma viruses in the retrovirus group shows interesting parallels. Murine sarcoma virus P60 (26), avian virus MC29 P110 (27), and feline sarcoma virus FOCMA antigen (90,000–100,000 molecular weight) (28) all contain some structural homology to the 5′ end of the *gag* gene of the original helper virus from which they were isolated. Thus, oncogenic retroviruses may often arise from fusion of the 5′-terminal portion of the *gag* region with other genetic material.

Although P120 is quite possibly responsible for fibroblast transformation by A-MuLV, the evidence for a helper function in A-MuLV lymphoid cell transformation implies that P120 is not sufficient to explain lymphoid transformation. Because no M-MuLV protein was evident in nonproducer lymphoid transformants, a role for the helper in maintenance of the transformed state is not indicated and it may be needed only for establishing transformation.

In cells producing A-MuLV and helper MuLV, the P120 is very stable intracellularly during pulse-chase experiments with a half-life of 3–6 hr. Only a small fraction can be recovered in the supernatant medium and only a fraction of this is found in the particulate fraction containing virus (unpublished data). No defined cleavage products of P120 are seen to accumulate intracellularly but only those retaining MuLV antigenicity could be recovered in these experiments. Preliminary cell fractionation studies have recovered a large fraction (80–90%) of P120 in membrane-bound form but we are as yet uncertain how much, if any, is found on the cell surface.

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1. Abelson, H. T. & Rabstein, L. S. (1970) *Cancer Res.* **30,** 2213–2222.
2. Rosenberg, N. & Baltimore, D. (1976) *J. Exp. Med.* **143,** 1453–1463.
3. Pratt, D. M., Strominger, J., Parkman, R., Kaplan, D., Schwaber, J., Rosenberg, N. & Scher, C. D. (1977) *Cell* **12,** 683–690.
4. Prekumar, E., Potter, M., Singer, P. A. & Sklar, M. D. (1975) *Cell* **6,** 149–159.
5. Potter, M., Sklar, M. D. & Rowe, W. P. (1973) *Science* **182,** 592–594.
6. Sklar, M. D., Shevach, E. M., Green, I. & Potter, M. (1975) *Nature* **253,** 550–552.
7. Silverstone, A. E., Rosenberg, N., Sato, V. L., Scheid, M. P., Boyse, E. A. & Baltimore, D. (1978) *Control of Cell Proliferation* (Cold Spring Harbor Press, Cold Spring Harbor, NY), Vol. 5, in press.
8. Scher, C. D. & Siegler, R. (1975) *Nature* **253,** 729–731.
9. Parks, W. P., Hawk, R. S., Anisowicz, A. & Scolnick, E. M. (1976) *J. Virol.* **18,** 491–503.
10. Rosenberg, N. & Baltimore, D. (1978) *J. Exp. Med.* **147,** 1126–1141.
11. Scher, C. D. (1978) *J. Exp. Med.* **147,** 1044–1053.
12. Haimovich, J., Bergman, Y., Linker-Israeli, M. & Haran-Ghera, N. (1977) *Eur. J. Immunol.* **7,** 226–230.
13. Arnstein, P., Riggs, J. L., Oshiro, L. S., Huebner, R. J. & Lennette, E. H. (1976) *J. Natl. Cancer Inst.* **57,** 1085–1090.
14. Pelham, H. R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67,** 247–256.
15. Philipson, L., Andersson, P., Olshevsky, U., Weinberg, R., Baltimore, D. & Gesteland, R. (1978) *Cell* **13,** 189–199.
16. Witte, O. N. & Baltimore, D. (1978) *J. Virol.*, in press.
17. Kessler, S. W. (1975) *J. Immunol.* **115,** 1617–1624
18. Witte, O. N. & Weissman, I. L. (1974) *Virology* **61,** 575–587.
19. Laemmli, U. K. (1970) *Nature* **227,** 680–685.
20. Witte, O. N., Tsukamoto-Adey, A. & Weissman, I. L. (1977) *Virology* **76,** 539–553.
21. Bonnar, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83–88.
22. Barbacid, M., Stephenson, J. R. & Aaronson, S. A. (1976) *Nature* **262,** 554–559.
23. Jamjoom, G. A., Naso, R. B. & Arlinghaus, R. (1977) *Virology* **78,** 11–34.
24. Bassin, R. H., Tuttle, N. & Fischinger, P. J. (1971) *Nature* **269,** 564–566.
25. Rothenberg, E., Donoghue, D. J. & Baltimore, D. (1978) *Cell* **13,** 435–451.
26. Robey, W. G., Oskarrsson, M. K., Van de Woude, G. F., Naso, R. B., Arlinghaus, R. B., Haapala, D. & Fischinger, P. J. (1977) *Cell* **10,** 79–89.
27. Bister, K., Hayman, M. J. & Vogt, P. K. (1977) *Virology* **82,** 431–448.
28. Stephenson, J. R., Khan, A. S., Sliski, A. H. & Essex, M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5608–5612.