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Analysis of v-*mos* Encoded Proteins in Cells Transformed by Several Related Murine Sarcoma Viruses

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We have used antisera against synthetic peptides to identify and characterize a 37,000 dalton v-*mos* encoded protein (p37*mos*) in cells transformed by M-MuSV 124. p37*mos*, a phosphoprotein, comprises only about 0.0005% of total cellular protein in cell lines transformed by M-MuSV 124. NIH 3T3 cells acutely infected with M-MuSV 124, however, contain 30–100-fold more p37*mos*. These elevated levels of p37*mos* correlate with striking morphological changes and cell death in the acutely infected cell population. Using the antipeptide antisera, we have extended the analysis of v-*mos* proteins to include several other MuSV variants that contain a similar v-*mos* gene to M-MuSV 124. With the exception of P85, the *gag-mos* fusion protein from ts110 MuSV, the v-*mos* gene of these variants is expressed as a 35,000–37,000 dalton protein (size depending on the particular virus).

Key words: synthetic peptide antiserum, retrovirus, v-*mos*, Moloney murine sarcoma virus (MuSV)

Two different murine sarcoma viruses which contain v-*mos* sequences have been isolated from mice. The first isolate, Moloney murine sarcoma virus (M-MuSV), was derived from a fibrosarcoma which arose in a BALB/c mouse that had been injected with Moloney murine leukemia virus (M-MuLV) [1]. Like many other oncogenic retroviruses, the M-MuSV genome arose by a complex recombination event between the nonsarcomagenic M-MuLV genome and normal mouse cellular sequences, c-*mos* [2–7]. In addition, portions of the viral structural genes were deleted from the MuSV genome [8,9]. Transfection experiments with MuSV-derived DNA fragments demonstrated that the acquired cellular sequences, v-*mos*, within the M-MuSV genome contain sufficient information to induce malignant transformation of fibroblasts in vitro [10-14]. The cellular gene, c-*mos*, from which the v-*mos* gene was derived is also capable of transforming cells when transfected together with a viral control element [15].

In addition to M-MuSV clone 124 and its subclone M-MuSV clone 349 [16], several other MuSV variants have been derived at different times during serial passage of the original M-MuSV producing tumor stock in animals or tissue culture cells. The

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variants all retain a similar v-*mos* gene but have major differences in the residual structural gene sequences [8]. These variants are denoted HT1-MuSV [17], NP-MuSV [18], ml-MuSV [19], and myeloproliferative sarcoma virus (MPSV) [20]. MPSV is of particular interest since, unlike the other M-MuSV variants that only transform fibroblasts, it is capable of transforming both hematopoietic cells as well as fibroblasts [20]. A mutant that is temperature-sensitive for maintenence of transformation, ts110-MuSV, was derived after UV irradiation of wild type M-MuSV clone 349 followed by a selection procedure that is toxic to cells that grow in agar at 39°C [21]. Cells infected with ts110-MuSV are transformed at 32°C but rapidly revert at 39°C to a normal state.

The second reportedly independent v-*mos* containing MuSV isolate, Gazdar MuSV (Gz-MuSV), was derived from a spontaneous tumor in a NZW/NZB F_1 hybrid mouse [22,23] and appears to have acquired the same cellular gene as M-MuSV [8]. No variants or temperature sensitive mutants of this isolate have been described.

In order to identify v-*mos* encoded proteins, virion RNA from M-MuSV 124, the most extensively characterized v-*mos* containing sarcoma virus, was used for in vitro translation experiments [24–28]. These experiments demonstrated that the v-*mos* sequences within M-MuSV 124 virion RNA could be translated in vitro to yield an overlapping set of four proteins of approximately 37K, 33K, 24K, and 18K [24, 25]. The structure of the 37K in vitro translation product is consistent with that of a 41K protein predicted from the single long open reading frame in the v-*mos* nucleotide sequence [25]. The three smaller in vitro products of 33K, 24K, and 18K are initiated from AUG codons within the 37K open reading frame [7, 24, 25, 29–32]. All four in vitro v-*mos* proteins share a commmon COOH-terminus and can be precipitated with an antiserum raised against a synthetic peptide corresponding to the COOH-terminal 12 amino acids of the protein predicted from the open reading frame in the M-MuSV 124 v-*mos* nucleotide sequence [25]. This paper summarizes the results of experiments in which the antipeptide serum was used to screen cells transformed by the MuSV isolates described above for the presence of v-*mos* related proteins.

MATERIALS AND METHODS

Antisera

The production of antisera against the peptides C1, C2, and C3, which correspond to the predicted COOH-terminal 6, 8, and 12 amino acids of the v-*mos* gene products respectively, has been described elsewhere [25]. Where indicated the antiserum was affinity purified by passage over a resin to which the C3 peptide had been covalently coupled. The IgG content of the affinity purified serum was 200 μg/ml.

Cell Lines

All cells were grown in Dulbecco's modified Eagle's medium (DME) with 5% calf serum. The M-MuSV 124 transformed producer NIH 3T3 cells and cDNA transformed nonproducer NIH 3T3 cells (N2) were derived as described [33]. M-MuSV 124 is a clonal isolate from the original M-MuSV tumor stock [16]. HT1-MuSV transformed nonproducer hamster cells, Gz-MuSV transformed producer rat cells and ml-MuSV transformed nonproducer mouse cells were kindly provided by D. Haapala. MPSV transformed producer and nonproducer rat cells (clones 66-3 and

p5-8) were obtained from W. Ostertag. R. Arlinghaus kindly provided the ts110-MuSV infected nonproducer rat cells (6m2) and producer rat cells. The ts110 infected cells were grown at either 32°C, the permissive temperature for transformation, or at 39°C, the nonpermissive temperature. All other cell lines were grown at 37°C.

NIH 3T3 cells were infected with M-MuSV 124 by preincubating cells for 4 hr with standard medium containing 10 μg/ml Polybrene, followed by a 2-hr incubation with filtered medium from the M-MuSV 124 transformed producer cells made up to 10 μg/ml with Polybrene. The virus containing medium was analyzed by focus assay and a sufficient amount was used to give a multiplicity of infection of 3–5 ffu/cell. After M-MuSV 124 infection the cells were cultured in standard medium that was changed every 3–4 days. NIH 3T3 cells were infected in similar manner with Gz-MuSV or with ts110-MuSV (harvested at 32°C).

Labeling of Cells and Immunoprecipitation

Cells grown in 35-mm dishes (approximately 80% confluent) were labeled for 2.5 hr at 32°C/39°C (ts110-MuSV infected cells) or at 37°C (all other cells). The labeling medium consisted of 0.5 ml DME lacking either methionine or phosphate containing either 0.25 mCi ^{35}S-methionine (Amersham) or 0.25 mCi $^{32}PO_4$, respectively. After the labeling period, cells were washed once with Tris-buffered saline and solubilized in 0.5 ml RIPA buffer (0.15 M NaCl, 0.01 M sodium phosphate pH 7.0, 1% Nonidet-P40, 1% sodium deoxycholate, 0.1% SDS, and 1% Trasylol) for 15 min at 4°C. The cell lysates were scraped from the dish and centrifuged in a Beckman JA20.1 rotor at 15,000 rpm for 1 hr at 4°C. The supernatant was divided into two 0.25-ml aliquots. One aliquot was immunoprecipitated with 1 ul of antipeptide serum and the other with 1 μl of antipeptide serum preabsorbed with 3 μg C3 peptide. Titration experiments showed that 1 μl of anti-C3 serum was an excess for the amount of p37*mos* in one half of an 80% confluent 35-mm dish of stable M-MuSV 124 transformed cells. For experiments in which acutely infected cells were immunoprecipitated in parallel with the stable cell lines, 6 μl of anti-C3 serum or 6 μl of anti-C3 serum preabsorbed with 10 μg C3 peptide was used (determined to be in excess) for all cell lysates. For the experiment shown in Figure 4, aliquots of the cell lysates were taken such that an equal number of ^{35}S-methionine cpm were subjected to immunoprecipitation for each cell type. The immunoprecipitates were collected by absorption to S. aureus bacteria, pelleted through 1 ml of RIPA containing 10% sucrose, and washed four times with 1 ml RIPA. The immunoprecipitate was solubilized in sample buffer. A portion of each immunoprecipitate corresponding to 1 ul of antiserum was analyzed by SDS polyacrylamide gel electrophoresis.

In Vitro Translation

In vitro translation of MuSV virion RNA was carried out as described [24, 25]. One μl of total in vitro translation product was analyzed by SDS polyacrylamide gel electrophoresis.

Gel Electrophoresis

SDS-polyacrylamide gels containing 12.5% acrylamide and 0.1% bisacrylamide were run as described previously [24]. Radioactive proteins were detected by fluorography.

Peptide Mapping Experiments

Two 9-cm dishes of M-MuSV 124 transformed cells were labeled with 2.5 ml per dish of medium containing 5 mCi ^{35}S-methionine. Immunoprecipitation with 250 μl of affinity purified serum was carried out as described above. The 37K M-MuSV 124 in vitro translation product was prepared and two dimensional chymotryptic peptide mapping was carried out as described [24, 34].

RESULTS AND DISCUSSION

In order to identify proteins potentially encoded by the v-*mos* gene, several MuSV transformed cell lines were immunoprecipitated with the anti-C3 serum described above. From the results of preliminary immunoprecipitation experiments, it was not possible to distinguish v-*mos* related proteins from the many nonspecifically precipitated cellular proteins. As we subsequently discovered, this difficulty was due in part to the scarcity of v-*mos* proteins in MuSV transformed cell lines. The following strategy was adopted to distinguish v-*mos* proteins from the other proteins seen in the immunoprecipitates. Since an excess of soluble C3 peptide abrogates the precipitation by anti-C3 serum of the v-*mos* in vitro translation products [25], it seemed reasonable to expect that the immunoprecipitation of v-*mos* related proteins from cells could also be specifically blocked with C3 peptide. In this approach, each cell lysate is divided into two parts and one portion is precipitated with anti-C3 serum (the "A" tracks in every figure) while the other is precipitated with anti-C3 serum preabsorbed with an excess of C3 peptide (the "B" tracks in every figure). In this manner, a 37,000-dalton protein doublet (p37) was identified in the anti-C3 serum immunoprecipitates from M-MuSV 124 transformed NIH 3T3 producer cells labeled for 2.5 hr with ^{35}S-methionine (Fig. 1). The p37 doublet was the only component that could be reproducibly blocked from the anti-C3 immunoprecipitate by C3 peptide (Fig. 1). The lower band in this p37 doublet comigrated with the 37K M-MuSV 124 in vitro translation product (Fig. 1). The same ^{35}S-methionine-labeled p37 doublet can be immunoprecipitated by anti-C3 serum from M-MuSV 124 cDNA transformed nonproducer cells (Fig. 1) that harbor only a single copy of the v-*mos* gene [33]. Anti-C3 serum did not detect any proteins similar to the 33K, 24K, and 18K in vitro v-*mos* products in M-MuSV 124 transformed cells (Fig. 1). By the criteria outlined above, none of the proteins present in immunoprecipitates made from uninfected NIH 3T3 cells (Fig. 1) or M-MuLV infected cells (data not shown) is recognized specifically by anti-C3 serum.

To show conclusively that p37 was encoded by the v-*mos* gene of M-MuSV 124, the chymotryptic peptide map of the p37 doublet was compared to the chymotryptic map of the 37K in vitro translation product, which we previously showed to be coded for by the M-MuSV 124 v-*mos* gene [24,25]. Figure 2 shows that the methionine-containing chymotryptic peptide maps of the two proteins are almost identical. This was confirmed by an analysis of mixtures of the two chymotryptic digests [34].

Immunoprecipitation with anti-C3 serum of the M-MuSV 124 transformed NIH 3T3 producer cells labeled with ^{32}P-orthophosphate revealed a single, specifically precipitated phosphoprotein with a similar mobility to the upper band of the ^{35}S-methionine-labeled doublet (Fig. 1). Both the ^{35}S-methionine-labeled doublet and the ^{32}P-labeled species will be referred to as p37*mos*. Phosphoaminoacid analysis and

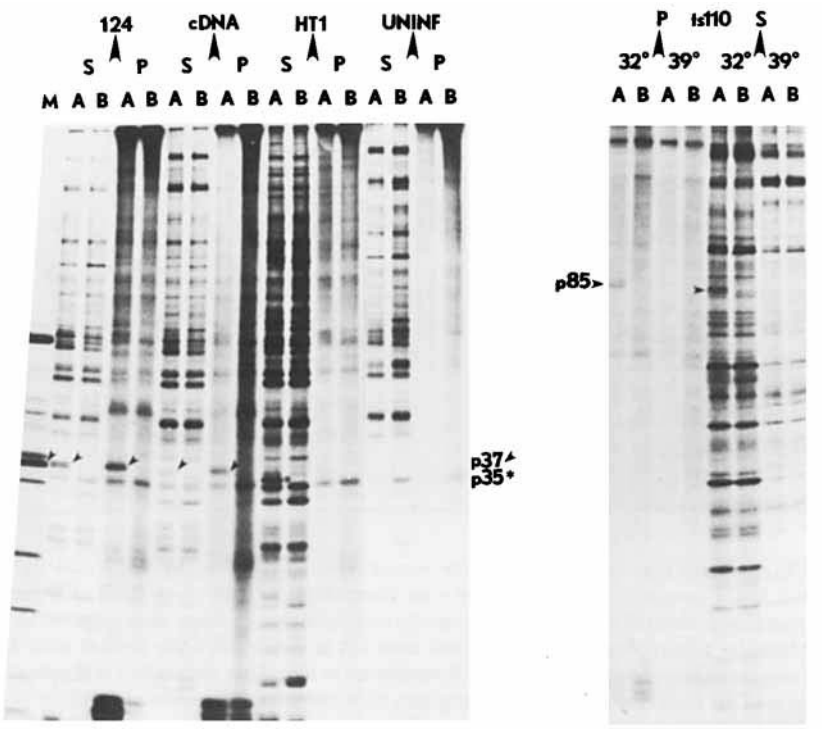

Fig. 1. Immunoprecipitation of cells with anti-C3 serum. Cells grown in 35-mm dishes were labeled for 2.5 hr with either ^{35}S-methionine (pairs of tracks labeled "S") or ^{32}P-orthophosphate (pairs of tracks labeled "P") and immunoprecipitation was carried out as described in Materials and Methods. A clarified lysate from a single dish of each cell type was divided into two equal aliquots. One aliquot was immunoprecipitated with 1 μl of anti-C3 serum (tracks A) and the other with 1 μl of anti-C3 serum pre-absorbed with 3 μg C3 peptide (tracks B). The entire immunoprecipitate was analyzed on a 12.5% SDS-polyacrylamide gel. The gel was fluorographed and exposed to film for 7 days (left panel) or 21 days (right panel). The cell lines used for immunoprecipitation are labeled as follows: a line of producer NIH 3T3 cells transformed with M-MuSV 124, 124; a nonproducer cell line transformed with M-MuSV 124 cDNA, cDNA; a nonproducer cell line transformed with HT1-MuSV, HT1; an uninfected NIH 3T3 cell line, UNINF; a producer cell line transformed with ts110-MuSV grown at the permissive temperature (32°C) or the nonpermissive temperature (39°C), ts110. p37 from M-MuSV 124 is denoted with an arrow and p35 from HT1-MuSV transformed cells is marked with a star. In the righthand panel, P85 from ts110-MuSV transformed cells is denoted by an arrow. The track labeled "M" contains a total in vitro translation product from M-MuSV 124 virion RNA with the position of the 37K v-*mos* protein marked with an arrow.

chymotryptic peptide mapping show that p37*mos* contains at least six sites of serine phosphorylation [34]. Since pulse-chase studies show that the lower band in the ^{35}S-doublet is the primary translation product that is subsequently converted to the upper band [34], which in turn comigrates with the ^{32}P-labeled p37*mos*, this conversion is likely to be due to extensive phosphorylation.

The immunoprecipitation experiments shown in Figure 1 were performed with an antiserum against a peptide (anti-C3) consisting of the COOH-terminal 12 amino acids predicted from the M-MuSV 124 nucleotide sequence. We have also screened

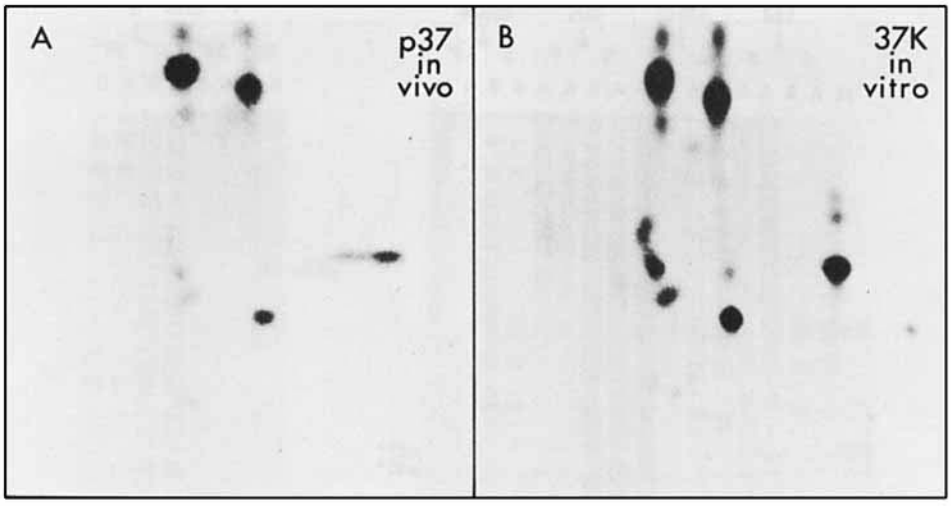

Fig. 2. Chymotryptic peptide maps of p37*mos* immunoprecipitated from M-MuSV 124 transformed cells and the 37K v-*mos* protein synthesized in vitro from M-MuSV 124 virion RNA. ^{35}S-methionine-labeled proteins were isolated and two dimensional chymotryptic peptide maps were prepared as in Materials and Methods. Electrophoresis at pH 4.7 was from left to right toward the cathode with the origin on the left and ascending chromatography was from bottom to top. Approximately 3,000 cpm of protein digest were used for each map and the chromatograms were coated with 2-methylnaphthalene containing 0.4% PPO and exposed to film for 30 days. Panel A shows a chymotryptic peptide map of the combined p37 doublet prepared from a cell line transformed with M-MuSV 124. Panel B shows a chymotryptic peptide map of the 37K v-*mos* protein synthesized in vitro from M-MuSV 124 virion RNA.

M-MuSV 124 transformed cells with antisera against shorter peptides consisting of the predicted COOH-terminal six (anti-C1) or eight (anti-C2) amino acids of the v-*mos* protein. As shown in Figure 3, anti-C1 and anti-C2 sera are capable of immunoprecipitating p37*mos* from M-MuSV 124 transformed cells with an efficiency roughly equal to that of anti-C3 serum. We also attempted to raise antisera against various predicted p37*mos* NH_2-terminal peptides (with/without acetylation of the NH_2-terminal residue, with/without the NH_2-terminal methionine). However, none of these peptides elicited antibodies which recognized p37*mos* (data not shown).

The combined p37*mos* doublet from M-MuSV 124 transformed cells comprises between 0.0002% and 0.0008% of cellular protein (expressed as a fraction of the total radioactivity subjected to immunoprecipitation) [34]. This corresponds to 10,000 to 40,000 molecules per cell, a value that is several orders of magnitude less than for other retroviral transforming proteins. Therefore, as mentioned previously, p37*mos* is a scarce protein in M-MuSV transformed cell lines. In contrast, immunoprecipitation of NIH 3T3 cells acutely infected by M-MuSV 124 revealed a much higher level of ^{35}S-methionine or ^{32}P-labeled p37*mos* (Fig. 4). Depending on the experiment, approximately 30–100-fold more p37*mos* could be precipitated from NIH 3T3 cells harvested 60 hr after infection with M-MuSV 124 at a multiplicity of 3–5 ffu per cell when compared to an equal amount of radioactively labeled cell lysate from the M-MuSV 124 transformed cell line [34]. No p37*mos* could be detected when NIH 3T3

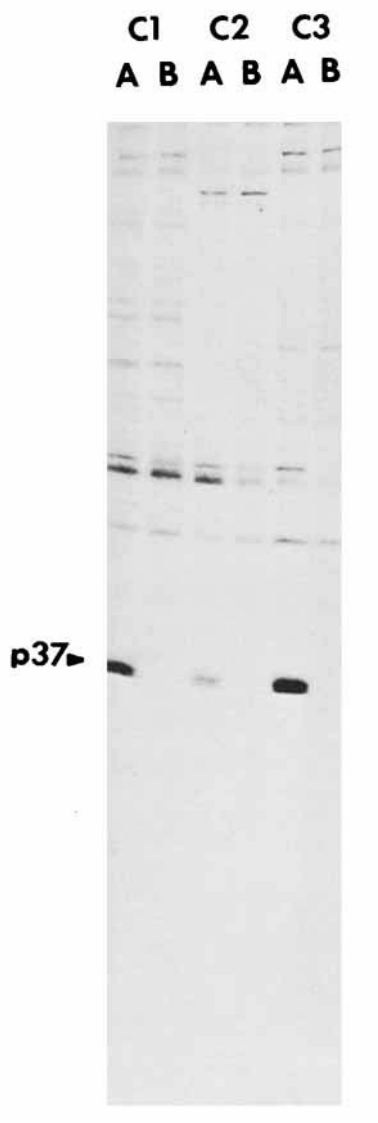

Fig. 3. Immunoprecipitation of NIH 3T3 cells acutely infected with M-MuSV 124 with different anti-peptide antisera. Three 35-mm dishes containing approximately equal numbers of NIH 3T3 cells 4 days after acute infection with M-MuSV 124 were labeled with ^{35}S-methionine. The cell lysate from each dish was divided into two equal aliquots. The first aliquot of each pair was immunoprecipitated with 3 μl of antipeptide antiserum (tracks A) and the second aliquot with 3 μl antipeptide serum preabsorbed with 10 μg C3 peptide (tracks B). The first dish was immunoprecipitated with anti-C1 peptide serum ("C1"), the second with anti-C2 peptide serum ("C2") and the third with anti-C3 peptide serum ("C3"). One-half of each immunoprecipitate was analyzed on a 12.5% SDS-polyacrylamide gel. The gel was fluorographed and exposed to film for 5 days.

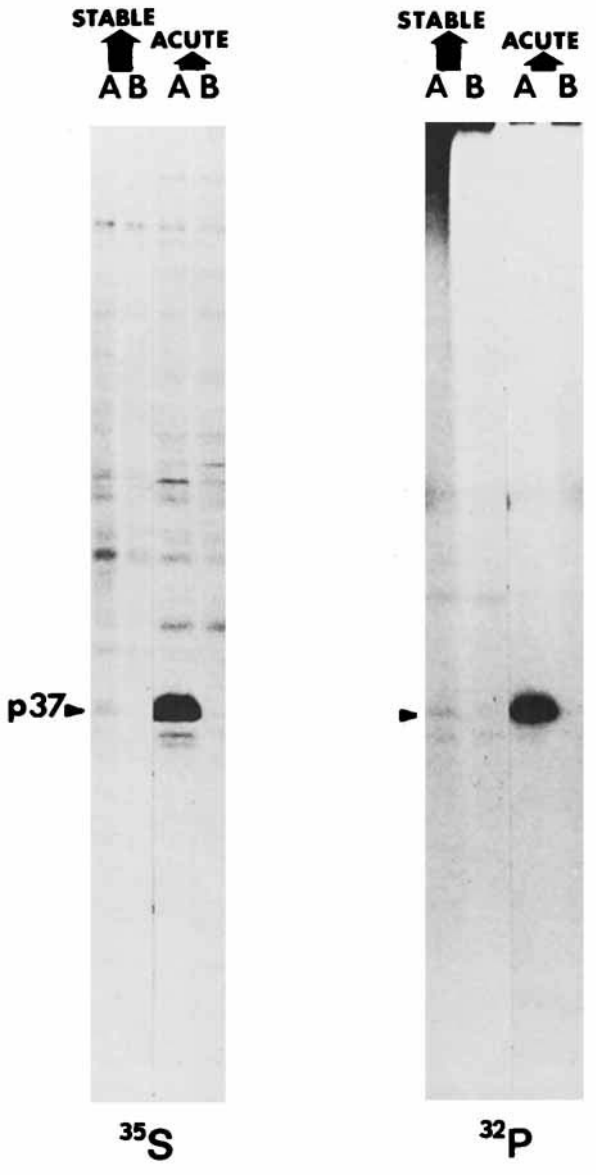

Fig. 4. Relative amounts of p37*mos* immunoprecipitated from a stable cell line transformed with M-MuSV 124 and from cells acutely infected with M-MuSV 124. 35-mm dishes of both a stable cell line transformed with M-MuSV 124 and NIH 3T3 cells acutely infected with M-MuSV 124 60 hr postinfection were labeled with ^{35}S-methionine (panel "^{35}S") or ^{32}P-orthophosphate (panel "^{32}P") as outlined in Materials and Methods. Two aliquots were taken from the clarified cell lysate for each sample so that an equal number of cpm were subjected to immunoprecipitation for each cell type. The first aliquot was precipitated with 6 μl anti-C3 serum (tracks A) and the second aliquot with 6 μl anti-C3 serum preabsorbed with 10 μg C3 peptide (tracks B). One-third of each immunoprecipitate was analyzed on a 12.5% SDS-polyacrylamide gel. The gel was fluorographed and exposed to film for 4 days (^{35}S panel) and 18 hr (^{32}P panel). The tracks corresponding to immunoprecipitates from NIH 3T3 cells acutely infected with M-MuSV 124 are labeled "acute" and the tracks corresponding to immunoprecipitates from a stable M-MuSV 124 transformed cell line are labeled "stable".

cells were acutely infected with MuLV or mock infected with tissue culture medium from M-MuSV transformed nonproducer cells (data not shown).

The synthesis of p37*mos* was first detectable at approximately 16 hr after infection of NIH 3T3 cells with M-MuSV 124. The level of p37*mos* continued to increase reaching a maximum at around 42 hr after infection. At this point, the amount of p37*mos* detectable in this cell population was 30–100-fold higher than that found in the stable transformed cell line, The level of p37*mos* remained high until approximately 7 days after infection when it began to decline. By 14 days after infection, the amount of detectable p37*mos* was approximately the same as the level found in the stable M-MuSV 124 transformed cell line [34].

During the course of these experiments it became clear that the appearance, plateau and decline in the synthesis of p37*mos* paralleled striking morphological changes and cell death in the acutely infected cell population (see Fig. 5). First, complete morphological transformation of the cells immediately followed the appearance and initial increase in p37*mos*. During the plateau phase (42 hr to 7 days after infection), when levels of p37*mos* remained high, the cells formed an increasingly disordered array and many became progressively more elongated with extremely long apical processes. Other cells, with 3–5 nuclei, were flat with many fingerlike projections. By 8-9 days after infection, approximately 80% of the cells were no longer adherent and floated in the medium as either single cells or multicellular clumps. Trypan blue staining showed that approximately 50% of the cells were nonviable. These nonattached cells did not incorporate ^{35}S-methionine, presumably because they were dead or dying, and could not be analyzed for the levels of p37*mos*. The adherent cells that multiplied slowly consisted of a mixed population that included many morphologically untransformed cells as well as cells that appeared transformed but were much less elongated and rounded up than those present between days 2 and 7. These transformed cells were morphologically very similar to the M-MuSV 124 transformed mouse cell lines. Furthermore, the population of cells that grew out by 14 to 21 days (termed "recovered") had a low level of p37*mos* similar to that in the stable M-MuSV 124 transformed cell line [34]. One interpretation of the correlation between elevated levels of p37*mos* and cell death is that p37*mos* is lethal to cells at high levels. Stable MuSV transformed cell lines may represent survivors that have been selected to express low amounts of p37*mos*. Extensive cell killing and chromosome damage after M-MuSV infection of 3T3 cells has previously been observed [35 and Fischinger, personal communication]. Furthermore, high levels of viral DNA and/or viral gene products has been correlated with a lethal effect for spleen necrosis virus [36], Rous sarcoma virus [37], and Abelson murine leukemia virus [38].

The experiments described above demonstrate that the anti-C3 serum is capable of recognizing both the in vitro and in vivo v-*mos* proteins from M-MuSV 124. It was of interest, therefore, to determine whether this antiserum could be used to identify v-*mos* proteins encoded by other viruses containing v-*mos* sequences. The results of these experiments are summarized in Table I and are discussed in detail below. Anti-C3 serum specifically recognizes a single ^{35}S-methionine labeled protein of approximately 35K (p35*mos*) in nonproducer hamster cells transformed by HT1-MuSV (Fig. 1). The ^{35}S-methionine containing chymotryptic peptide map of p35*mos* is almost identical to that of p37*mos* immunoprecipitated from M-MuSV 124 transformed cells [34]. The abundance of p35*mos* in HT1-MuSV transformed hamster cells was similar to that of p37*mos* in M-MuSV 124 transformed mouse cells [34].

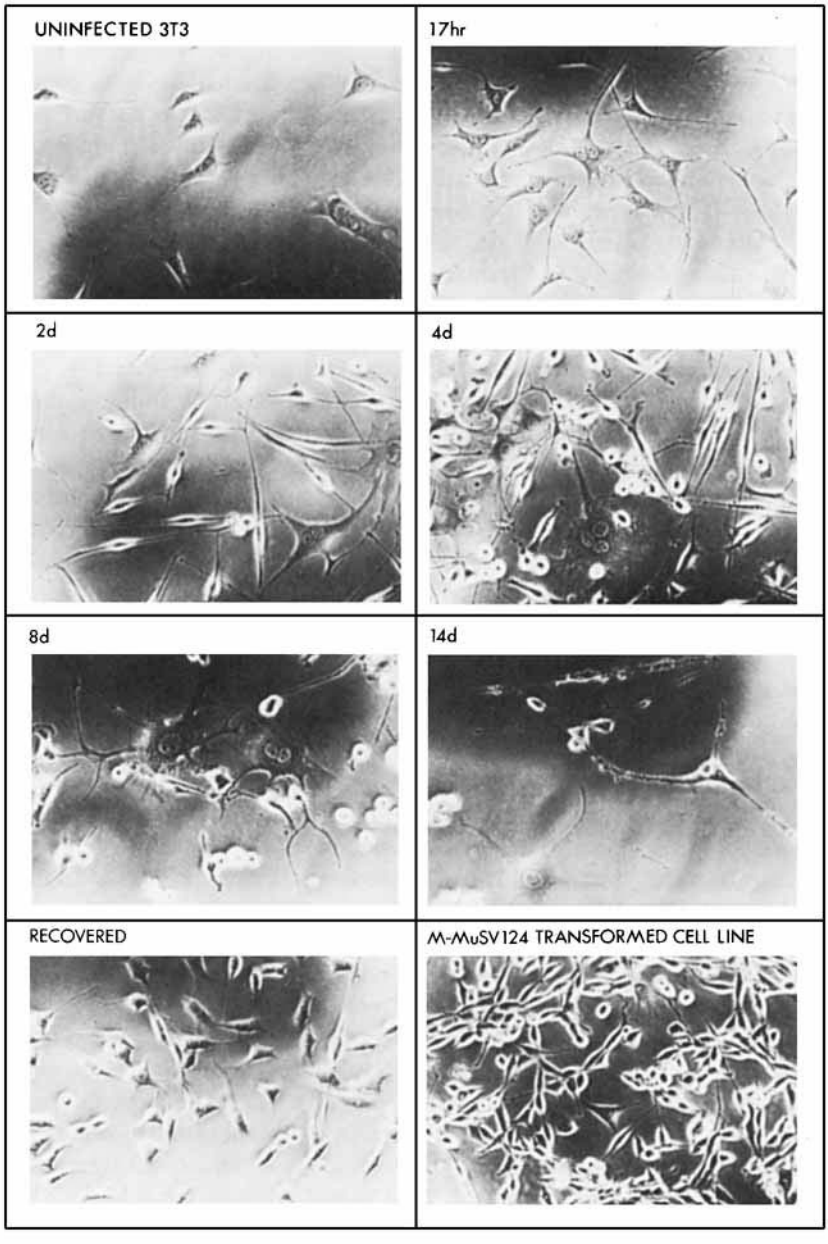

Fig. 5. Photographs of cells at various times after acute infection with M-MuSV 124. 35-mm dishes of NIH 3T3 cells were infected with 3–5 ffu of M-MuSV 124. Acute infections were initiated at different times such that representative cells at various times after infection were all available on the same day for photography. Cells were examined and photographed using an Olympus photomicroscope with Kodak Panomatic-X film at × 200. Photographs of cells at 17 hr, 2 days, 4 days, 8 days, and 14 days after acute infection are shown. Photographs are also shown of uninfected NIH 3T3 cells, a stable cell line transformed with M-MuSV 124 and a representative population of cells that survived M-MuSV infection and appeared at around 14–21 days postinfection (marked "recovered").

TABLE I. Properties of v-*mos* Proteins From Various Strains of MuSV

Virus strain	Transformed cell type	Largest in vitro v-*mos* product[a]	In vivo v-*mos* protein[b]	Phosphorylated?	Elevated levels after acute infection?
M-MuSV 124	Mouse producer	37K	p37	+++	+++
	Mouse cDNA transformed		p37	+++	
HT1-MuSV	Hamster nonproducer	35K[c]	p35	+/−	ND
NP-MuSV	Mouse nonproducer	ND	p35	+/−	ND
Gz-MuSV	Rat producer	37K	p37?	ND	−
ts110 MuSV	Rat producer	85K[d]	p85	++	+
	Rat nonproducer		p85	++	ND
ml-MuSV	Mouse nonproducer	ND	−	ND	ND
MPSV	Rat nonproducer	35K	−	ND	ND

Unless otherwise noted the results in this table are described or cited in this paper.
[a] v-*mos* in vitro translation products identified by immunoprecipitation with anti-C3 serum and by peptide mapping.
[b] v-*mos* proteins in transformed cells identified by immunoprecipitation with anti-C3 serum.
[c] Cremer et al [28].
[d] Horn et al [40, 41].
ND, not done.

The apparent size difference between HT1-MuSV p35*mos* and M-MuSV 124 p37*mos* is not reflected as a difference in the chymotryptic peptide maps although differences might have been missed because the methionine-containing chymotryptic peptides predicted from the M-MuSV 124 v-*mos* nucleotide sequence do not represent the entire protein sequence. The size difference between p35*mos* and p37*mos* is unlikely to be due to a post-translational processing event since the largest v-*mos* proteins synthesized in vitro from M-MuSV 124 virion RNA and HT1-MuSV virion RNA are 37K and 35K, respectively [24,25,28].

Unlike p37*mos* seen in M-MuSV 124 transformed mouse cells, the p35*mos* protein from the HT1-MuSV transformed hamster cells does not migrate as a doublet and is weakly phosphorylated if at all (Fig. 1). The heavily phosphorylated 34K band in the HT1-MuSV "P" tracks is not *mos*-specific since it is present in the blocked immunoprecipitate. This apparent lack of p35*mos* phosphorylation does not appear to be due to host cell differences since mouse cells transformed by NP-MuSV, a virus derived from HT1-MuSV [18], also contained very low levels of a single ^{35}S-methionine-labeled v-*mos* protein of 35K that was unphosphorylated (data not shown). These results imply that phosphorylation may not be necessary for the transforming activity of the v-*mos* protein. Alternatively, it is possible that only a single site of phosphorylation is necessary for biological activity and this site may be weakly, but sufficiently phosphorylated in p35*mos*.

Attempts to detect a v-*mos* protein by immunoprecipitation with anti-C3 serum from rat cells transformed by Gz-MuSV, rat cells transformed by MPSV or mouse cells transformed by ml-MuSV were largely unsuccessful (data not shown). This result was unexpected for Gz-MuSV since the 37K v-*mos* in vitro translation product from Gz-MuSV virion RNA, which is virtually identical to the 37K in vitro v-*mos* product from M-MuSV 124 by peptide mapping, can be immunoprecipitated with anti-C3 serum [39]. The most likely explanation for the apparent lack of a v-*mos* protein in Gz-MuSV transformed cells is that the level of p37*mos* is below the level

of detection in these cells. In fact, immunoprecipitation of rat cells transformed by Gz-MuSV with anti-C3 serum occasionally revealed, after very long exposures of the gel, an extremely faint specifically precipitated band of approximately 37K (data not shown).

On the other hand, our inability to immunoprecipitate a v-*mos* protein from MPSV transformed cells with anti-C3 serum is probably due to the fact that the COOH-terminus of the MPSV v-*mos* protein is different from that of M-MuSV 124, against which the serum is directed. This conclusion is supported by the fact that the largest v-*mos* in vitro translation product from MPSV virion RNA is approximately 35K and cannot be immunoprecipitated with anti-C3 serum [J.P., unpublished results]. The ml-MuSV v-*mos* product is also unlikely to be recognized by anti-C3 serum since recent nucleotide sequencing data shows that due to a small deletion the COOH-terminal 6 amino acids of the ml-MuSV v-*mos* product are entirely different from those of the M-MuSV 124 p37*mos* (Donoghue, personal communication).

Horn et al [40,41] have identified in cells transformed by ts110-MuSV an 85,000-dalton protein (P85) which appears, by peptide mapping, to be a *gag-mos* fusion product. Using an antiserum against the viral *gag* protein, P85 can be detected in ts110-infected cells maintained at 32°C, the permissive temperature for transformation, but not at 39°C, the nonpermissive temperature. Figure 1 demonstrates that anti-C3 serum specifically recognizes P85 from ^{35}S-methionine-labeled ts110-infected nonproducer cells grown at 32°C but not at 39°C. Anti-C3 serum also specifically precipitated an 85,000-dalton phosphoprotein from ^{32}P-labeled ts110-infected nonproducer rat cells grown at 32°C but not at 39°C (Fig. 1). In addition, P85 was immunoprecipitated from ^{35}S-methionine or ^{32}P-labeled ts110-infected producer rat cells at 32°C (data not shown). A similar result has been obtained by R. Arlinghaus and colleagues (personal communication) who used our anti-C3 serum to immunoprecipitate ts110-infected cell lysates. The amount of detectable P85 in these cell lines is slightly higher than the levels of p37*mos* seen in M-MuSV 124 transformed cell lines (see Fig. 2). Furthermore, when NIH 3T3 cells are acutely infected at 32°C with ts110 virus (harvested at 32°C) the level of P85*gag-mos* appears to be slightly elevated when compared to the stable ts110-transformed cell line (data not shown). ts110-MuSV is unique among the MuSV's in that it contains a v-*mos* gene that codes for a *gag-mos* fusion protein [40,41]. This can be accounted for by the unusual genomic structure revealed by heteroduplex mapping which shows that the 5′ two-thirds of the ts110 *gag* gene is juxtaposed to the 5′end of v-*mos* (Arlinghaus and Junghans, personal communication). The genomes of other MuSV isolates, however, contain various portions of *gag, pol,* or *env* gene sequences between *gag* and *mos* that presumably contain termination codons that would not allow the synthesis of a *gag-mos* fusion protein [8,9]. Since the v-*mos* region within the ts110 genome can be translated as part of a large *gag-mos* fusion product [40,41] with a COOH-terminus that is recognized by anti-C3 serum (Fig. 1), it is possible that the ts110 v-*mos* gene would also be expressed as a nonfused *mos* protein similar to p37*mos* from M-MuSV 124. In some experiments, NIH 3T3 cells acutely infected with ts110-MuSV appear to contain small quantities of an ^{35}S-methionine-labeled protein of approximately 33,000 daltons (p33) that is specifically recognized by the anti-C3 serum (data not shown). While definitive identification of p33 by peptide mapping has not been possible due to the scarcity of this protein, the immunoprecipitation results taken together with the fact that the largest in vitro v-*mos* product encoded by ts110 virion RNA that does not

contain *gag* sequences is 33K (Arlinghaus, personal communication) suggest that p33 may be a v-*mos* encoded product. Anti-C3 serum, however, does not specifically recognize any proteins other than P85*gag-mos* from ^{35}S-methionine or ^{32}P-labeled ts110 MuSV transformed producer or nonproducer cell lines (Fig. 1 and unpublished results). It is possible that p33 exists in all cells transformed by ts110 MuSV but its presence can only be revealed under conditions, such as acute infection, where the levels of v-*mos* products are elevated. Thus, P85*gag-mos* may not be the sole product from the ts110 v-*mos* gene in transformed cells.

In conclusion, the transforming gene, v-*mos*, carried by several related murine sarcoma viruses is expressed as a 35,000–37,000-dalton protein (size depending on the particular variant examined). The ts110 MuSV v-*mos* gene is expressed in a different manner as a 85,000-dalton *gag-mos* fusion protein. It is likely that the v-*mos* protein is directly involved in maintenance of the transformed state since P85*gag-mos* from ts110 MuSV is synthesized only at 32°C, the permissive temperature for transformation but not at 39°C, the nonpermissive temperature at which the cells appear to revert to normal phenotype. Furthermore, synthesis of p37*mos*, which can be identified in cells transformed by M-MuSV 124 cDNA (Fig. 1), decreases to undetectable levels when the cells revert morphologically. The function of the v-*mos* protein in transformation remains to be elucidated. Unlike the transforming gene products of several other retroviruses, neither the 37K M-MuSV 124 in vitro v-*mos* translation product nor p37*mos* from M-MuSV 124 transformed cells has protein kinase activity when measured by the standard immune complex assay (unpublished results). Furthermore, M-MuSV 124 transformed cell lines do not have elevated levels of phosphotyrosine [42], although cells acutely infected by M-MuSV 124 show a 1.5–2-fold increase in the level of total phosphotyrosine in protein (data not shown). This elevation is considerably smaller than that seen in cells transformed by retroviruses that code for tyrosine specific protein kinases [42] and its significance is unclear. Experiments are underway to identify the subcellular location of v-*mos* proteins in transformed cells. An understanding of the function of the v-*mos* gene will be useful in defining the possible function of the c-*mos* gene in normal cell growth and regulation.

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