Detection of an 85,000-Dalton Phosphoprotein in *ts110* Murine Sarcoma Virus-Infected Cells with Antiserum Against a *v-mos* Peptide

JACKIE PAPKOFF†* AND TONY HUNTER

Tumor Virology Laboratory, The Salk Institute, San Diego, California 92138

Received 12 August 1982/Accepted 2 December 1982

We have used an antiserum directed against a synthetic *v-mos* peptide (anti-C3 serum) to screen *ts110* murine sarcoma virus (MuSV)-infected cells for the presence of *v-mos*-encoded proteins. Anti-C3 serum specifically recognized an 85,000-dalton protein doublet (P85) from [³⁵S]methionine-labeled *ts110* MuSV-infected producer cells grown at 32°C, the permissive temperature for transformation. The P85 doublet was also recognized by an antiserum directed against the viral *gag* protein p15. P85 was present but at 2- to 10-fold-lower levels in *ts110* MuSV-infected producer cells grown at 39°C, the restrictive temperature for transformation. The P85^{*gag-mos*} fusion product was the only *v-mos* protein reproducibly detected in this *ts110* MuSV-transformed cell line. Immunoprecipitation of ³²P-labeled cells with anti-C3 serum revealed that the upper band of the P85 doublet is phosphorylated, containing mostly phosphoserine and some phosphothreonine. Cells acutely infected with *ts110* MuSV contained slightly higher levels of P85 than did the *ts110* MuSV-infected producer cell line. Anti-C3 serum specifically recognized a 33,000-dalton protein (p33) in the acutely infected cells labeled with [³⁵S]methionine. p33 was present in trace amounts and may represent a previously unidentified *ts110* MuSV-encoded *v-mos* protein.

Moloney murine sarcoma virus (M-MuSV) (10), a replication-defective retrovirus, induces sarcomas in animals and transforms fibroblasts in vitro. The oncogenic information of M-MuSV is contained within a block of approximately 1,200 nucleotides, called *v-mos*, at the 3' end of the MuSV genome (1, 3, 5, 6, 16, 17, 20-23). We have used an antiserum against a synthetic COOH-terminal peptide predicted from the *v-mos* DNA sequence to demonstrate that the M-MuSV *v-mos* gene is expressed in M-MuSV clone 124 (M-MuSV 124)-transformed cell lines as a 37,000-dalton phosphoprotein (p37^{*mos*}) (15). p37^{*mos*} comprises only about 0.0005% of the total cellular protein in cell lines transformed by M-MuSV 124 (15). NIH 3T3 cells acutely infected with M-MuSV 124, however, transiently contain 30- to 100-fold more p37^{*mos*} (15). p37^{*mos*} contains at least six sites of serine phosphorylation and appears to be a soluble cytoplasmic protein (15). For comparative purposes we have begun to analyze *v-mos* proteins of several MuSV variants which contain *v-mos* genes closely related to that of M-MuSV 124.

ts110 MuSV, a mutant which is temperature

sensitive for maintenance of transformation, was derived after UV irradiation of wild-type M-MuSV clone 349 (a subclone of M-MuSV 124) followed by a selection procedure that is toxic to cells that grow in agar at 39°C (2). Cells infected with *ts110* MuSV are transformed at 32°C but revert rapidly to a normal state upon being shifted to 39°C (2). Horn and co-workers have used antisera against the viral *gag* protein p12 to detect two MuSV-specific proteins in *ts110* MuSV-infected cells (7, 8). One of these, P58 (a 58,000-dalton protein), contains only *gag*-related sequences and is equally abundant at permissive and restrictive temperatures (7, 8). P58 appears to be synthesized from *gag* sequences in one of the two types of genomic RNA present in *ts110* MuSV virions (8). In contrast to P58, the second protein, P85, is only detectable in *ts110* MuSV-infected nonproducer cells maintained at the permissive temperature (7, 8). Tryptic peptide mapping shows that P85 contains not only *gag*-specific peptides but also peptides which are homologous to those from presumptive *v-mos* in vitro translation products from M-MuSV 349 (7, 8, 11). Thus, P85 appears to be a *gag-mos* fusion protein and is a likely candidate for the transforming protein of *ts110* MuSV (7, 8). The expression of such a chimeric protein is consis-

† Present address: Department of Pharmacology, Stanford University Medical Center, Stanford, CA 94305.

FIG. 1. Immunoprecipitation of *ts110* MuSV-infected cells with anti-C3 serum and anti-p15 serum. (A) *ts110* MuSV-infected producer cells (NRK 206.2 IC) (8) were grown in 5-cm dishes at either 32 or 39°C. Each dish, at approximately 80% confluence, was labeled for 2.5 h at 32 or 39°C. The labeling medium consisted of 1.0 ml of Dulbecco modified Eagle medium lacking either methionine or phosphate and containing either 0.8 mCi of [^{35}S]methionine (Amersham Corp.) or 0.5 mCi of $^{32}\text{PO}_4$ (ICN), respectively. After the labeling period, the cells were washed once with Tris-buffered saline and solubilized for 15 min in 0.65 ml of RIPA buffer (0.15 M NaCl, 0.01 M sodium phosphate [pH 7.0], 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% Trasylol). The cell lysates were scraped from the dish and centrifuged in a Beckman JA 20.1 rotor at 15,000 rpm for 1 h at 4°C. The [^{35}S]methionine-labeled supernatants were divided into three parts, and the ^{32}P -labeled supernatants were divided into two parts. Samples of the cell lysates were taken such that an equal number of either [^{35}S]methionine or ^{32}P counts per minute from 32 and 39°C cell lysates were subjected to immunoprecipitation with each different serum. For the [^{35}S]methionine-labeled lysates, the first sample was immunoprecipitated with 2 μl of anti-C3 serum (15), the second sample was immunoprecipitated with 2 μl of anti-C3 serum preabsorbed with 5 μg of C3 peptide, and the third sample was immunoprecipitated with 2 μl of anti-p15 serum. For the ^{32}P -labeled cell lysates, the first sample was immunoprecipitated with 2 μl of anti-C3 serum, and the second sample was immunoprecipitated with 2 μl of anti-C3 serum preabsorbed with 5 μg of C3 peptide. The immunoprecipitates were collected by absorption to *Staphylococcus aureus* bacteria, pelleted through 1 ml of RIPA buffer containing 10% sucrose, and washed three times with 1 ml of RIPA buffer. Each immunoprecipitate was solubilized in 30 μl of sample buffer, and 15 μl was analyzed on a 12.5% sodium dodecyl sulfate-polyacrylamide gel as described previously (12). The gel was fluorographed and exposed to film for 48 h (lanes A

tent with the structure of the second type of genomic RNA present in *ts110* MuSV virions. Heteroduplex mapping shows that the 5' two-thirds of the *gag* gene has been juxtaposed to the 5' end of the *v-mos* gene in these RNA molecules, with the resultant loss of about 100 nucleotides at the 5' end of the *v-mos* gene (9). Since Arlinghaus and co-workers have used exclusively anti-*gag* antisera to identify P85 in *ts110* MuSV-infected cells, a smaller *v-mos* protein lacking *gag* sequences, related to *p37^{mos}* from M-MuSV 124, would have gone undetected. To confirm that the COOH-terminal portion of P85 is *v-mos* encoded and to examine *ts110* MuSV-infected cells for the presence of a unique *v-mos* product, we immunoprecipitated *ts110* MuSV-infected producer cells with the antiserum against a synthetic M-MuSV 124 *v-mos* COOH-terminal peptide (anti-C3 serum) (14, 15).

A doublet of approximately 85,000 daltons was the only protein specifically precipitated from [³⁵S]methionine-labeled, *ts110* MuSV-infected producer cells grown at 32°C (Fig. 1A, lane A). A similar protein can be immunoprecipitated from *ts110* MuSV-infected nonproducer cells (6m2 cells) grown at 32°C (13, 19). Precipitation of the P85 doublet was specifically blocked with an excess of soluble C3 peptide (Fig. 1A, lane B), the criterion by which we distinguish *v-mos*-encoded proteins from unrelated proteins in immunoprecipitates made with anti-C3 serum (15). We did not detect a 37,000-dalton protein, similar to *p37^{mos}* (see Fig. 3), in immunoprecipitates from *ts110* MuSV-infected producer cells. An antiserum against the murine leukemia virus *gag* protein p15 recognized a triplet in the region of P85 from these same *ts110* MuSV-infected producer cells in addition to several smaller *gag*-related proteins (Fig. 1A, lane C). The upper two bands of the triplet comigrated with the two P85 proteins recognized by anti-C3 serum. The smallest band in the triplet appears to be a murine leukemia virus *gag* protein, since it was also precipitated by anti-p10 serum which does not recognize P85 (data not shown). The anti-p15 immunoprecipitation results shown here confirm previous experiments by Horn et al. in which anti-p12 serum was used to immunoprecipitate P85 (7, 8). It is noteworthy that anti-p15 serum precipitates P85 about five times more efficiently than does anti-

C3 serum when both sera are used in excess. Attempts to improve the precipitation efficiency with anti-C3 serum by boiling the cell lysates in sodium dodecyl sulfate to expose the single antigenic determinant in every P85 molecule before immunoprecipitation were unsuccessful (J. Papkoff, unpublished data).

We estimated the abundance of P85 in *ts110* MuSV-infected cells by making the assumptions that the half-life of P85 is similar to that of total cellular protein and that the immunoprecipitation procedure brings down a major fraction of the P85 population, as we did previously for *p37^{mos}* (15). Depending on the antiserum and the experiment, P85 comprised approximately 0.003 to 0.01% of total methionine in cellular protein in *ts110* MuSV-infected producer cells grown at 32°C. Making a correction for the methionine content of the two proteins, this corresponds to about 5 to 15 times as many P85 molecules per cell as the number of *p37^{mos}* molecules per cell detected in an NIH 3T3 cell line transformed with M-MuSV 124 (15).

Immunoprecipitation, with anti-C3 serum, of [³⁵S]methionine-labeled, *ts110* MuSV-infected producer cells grown at 39°C, the nonpermissive temperature, revealed a specifically precipitated P85 doublet (Fig. 1A). Anti-p15 serum also recognized a P85 triplet similar to that seen at 32°C. The upper band of the P85 doublet identified with anti-C3 serum at 39°C migrated slightly faster than the upper band of the P85 doublet precipitated from cells grown at 32°C (Fig. 1B). The significance of this small but reproducible difference is unclear but might reflect differences in phosphorylation at the two temperatures. The amount of P85 detectable in cells grown at 39°C was variable depending on the experiment and ranged from 2- to 10-fold less than that detectable when the cells were grown at 32°C. The presence of P85 in *ts110* MuSV-infected producer cells at 39°C has been observed previously by Horn and co-workers (8). It contrasts with the situation found with *ts110* MuSV-infected nonproducer cells, in which no P85 is detected at the nonpermissive temperature either with anti-p12 serum (7, 8) or with anti-C3 serum (13, 19). The "leakiness" of P85 expression in *ts110* MuSV-infected producer cells grown at 39°C could well be due to the presence of a few wild-type revertant cells synthesizing P85 rather than to a

and B) or 11 h (lanes C). The immunoprecipitates shown in the ³⁵S panel and the ³²P panel are from separate experiments. In each case all of the samples were run side by side on a single gel. Lanes: A, anti-C3 immunoprecipitate; B, blocked anti-C3 immunoprecipitate; C, anti-p15 immunoprecipitate. (B) *ts110* MuSV-infected cells were labeled and immunoprecipitated as described above. All three samples were analyzed side by side on the same gel. The gel was fluorographed and exposed to film for 6 days (lane 1) or 2 days (lanes 2 and 3). Lanes: 1, anti-C3 immunoprecipitate made from ³²P-labeled, *ts110* MuSV-infected cells grown at 32°C; 2, anti-C3 immunoprecipitate made from [³⁵S]methionine-labeled, *ts110* MuSV-infected cells grown at 32°C; 3, anti-C3 immunoprecipitate made from [³⁵S]methionine-labeled, *ts110* MuSV-infected cells grown at 39°C.

low level of P85 expression in all of the cells, since in our hands the *ts110* MuSV-infected cell population maintained at 39°C consists of scattered transformed cells among a population of morphologically flat cells.

A single specifically precipitated phosphoprotein of about 85,000 daltons could be identified in anti-C3 serum immunoprecipitates from ^{32}P -labeled, *ts110* MuSV-infected cells grown at 32°C (Fig. 1A). P85 was also detected, but at lower levels, in ^{32}P -labeled, *ts110* MuSV-infected cells grown at 39°C (Fig. 1A). The ^{32}P -labeled P85 band comigrated with the upper band of the [^{35}S]methionine-labeled P85 doublet (Fig. 1B). This situation is identical to that seen with p37^{mos} in M-MuSV 124-transformed cells. In this case we demonstrated, by pulse-chase analysis, that the lower band of the p37^{mos} doublet was the primary translation product which was subsequently converted to the upper band, probably by extensive phosphorylation (15). Pulse-chase analysis with anti-C3 serum shows that P85 synthesis and processing proceed in a similar manner (J. Papkoff, unpublished data). Phosphoamino acid analysis demonstrated that P85 contains primarily phosphoserine with some phosphothreonine (Fig. 2). No phosphotyrosine was detectable in P85. P85 has the expected phosphoamino acid composition for a *gag-mos* fusion protein, since the M-MuSV 124 *gag* protein P62 contains mostly phosphoserine and some phosphothreonine, whereas p37^{mos} has at least six sites of serine phosphorylation (15).

The immunoprecipitation experiments described above did not reveal any specifically precipitated proteins smaller than P85 in *ts110* MuSV-infected producer cells. In particular, no protein the size of p37^{mos} was detectable (Fig. 1A and 3). Since *v-mos* proteins in all stable transformed cell lines that we have examined are scarce proteins (e.g., p37^{mos} comprises approximately 0.0005% of total methionine in cellular protein in an M-MuSV 124-transformed cell line [15]), it is conceivable that a smaller *v-mos* product might be below the level of detection in *ts110* MuSV-transformed cell lines. Since NIH 3T3 cells acutely infected with M-MuSV 124 transiently contain 30- to 100-fold more p37^{mos} than does the stable M-MuSV 124-transformed cell line (15), unique *ts110* MuSV-encoded *v-mos* proteins might be present at higher levels in cells acutely infected with *ts110* MuSV. The acute infection process with *ts110* MuSV proceeded in a manner similar to that described for M-MuSV 124 (15). Immunoprecipitation with anti-C3 serum of [^{35}S]methionine-labeled NIH 3T3 cells 4 days after acute infection at 32°C with *ts110* MuSV revealed, as expected, the P85 doublet (Fig. 3). A similar result was obtained when acutely infected cells were labeled with

$^{32}\text{P}_i$ (data not shown). In contrast to the 30- to 100-fold elevation of p37^{mos} seen after acute infection of NIH 3T3 cells with M-MuSV 124, the level of P85 synthesis after acute infection with *ts110* MuSV was increased only about twofold. The failure to obtain a large increment in P85 synthesis after acute infection could be a consequence of the lower temperature (32 versus 37°C) at which the acute infections are carried out or may simply reflect our technical difficulties with the acute infection process.

In addition to P85, immunoprecipitation of NIH 3T3 cells acutely infected with *ts110* MuSV with anti-C3 serum yielded very small quantities of a specifically precipitated [^{35}S]methionine-labeled protein of approximately 33,000 daltons (*p33*) (Fig. 3). *p33* is the only protein smaller than P85 which was reproducibly blocked from the anti-C3 immunoprecipitate with C3 peptide; there was no evidence of a 37,000-dalton protein (Fig. 3). Although 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 lacking *gag* sequences encoded by *ts110* MuSV virion RNA is 33,000 daltons (11, 19) suggest that *p33* may be a *v-mos*-encoded product. The presence of *p33* in acutely infected cells may be a conse-

FIG. 2. Phosphoamino acid analysis of P85. ^{32}P -labeled P85 was immunoprecipitated from *ts110* MuSV-infected producer cells grown at 32°C as outlined in the legend to Fig. 1. P85 was extracted from the sodium dodecyl sulfate-polyacrylamide gel, and phosphoamino acid analysis was carried out in two dimensions as described previously (18). The first dimension (pH 1.9) was with the anode at the left and the origin at the bottom right (off the figure). The second dimension (pH 3.5) was with the anode at the top. Marker phosphoserine (pSER), phosphothreonine (pTHR), and phosphotyrosine (pTYR) were located by staining. A 160-cpm amount of P85 was analyzed, and an indirect autoradiograph of the map is shown (4-day exposure).

FIG. 3. Immunoprecipitation with anti-C3 serum of NIH 3T3 cells acutely infected with *ts110* MuSV. Dishes (35 mm) of NIH 3T3 cells were infected with *ts110* MuSV by preincubation of the cells for 4 h at 32°C with standard medium containing 10 μ g of Polybrene per ml, followed by a 4-h incubation at 32°C with 2 ml of filtered medium (made up to 10 μ g/ml with Polybrene) from *ts110* MuSV producer cells grown at 32°C. After infection, the cells were grown in standard medium at 32°C. Four days after infection, the acutely infected cells were labeled with [35 S]methionine and immunoprecipitated with anti-C3 serum as described in the legend to Fig. 1. (To reach a comparable stage, acute infection with *ts110* MuSV at 32°C required about twice as long as infection with M-MuSV 124 at 37°C, presumably due to the temperature differential.) For comparison, a cell lysate containing an equal number of [35 S]methionine counts per minute from the

sequence of infection with spontaneously occurring defective viral genomes from which some or all of the *gag* sequences are deleted or the result of proteolysis of P85 when present at increased levels. On the other hand, p33 may exist in all cells transformed by *ts110* MuSV but only be detectable under conditions, such as acute infection, where the levels of *v-mos* products are elevated.

In conclusion, we have demonstrated, using an antiserum against a synthetic *v-mos* peptide, that the *ts110* MuSV-encoded *gag-mos* fusion protein, P85, contains *v-mos* sequences homologous to those of p37^{mos}, the *v-mos*-encoded protein from M-MuSV 124. Stanker et al. have recently obtained very similar results with our anti-C3 serum (19). P85 is a phosphoprotein containing mostly phosphoserine and some phosphothreonine. We estimate that P85 comprises approximately 0.003 to 0.01% of total methionine in cellular protein in *ts110* MuSV-infected producer cells grown at 32°C. This level is about 5- to 15-fold higher than that observed for p37^{mos} in M-MuSV-transformed producer cells. On the basis of the cytopathic effects of acute infection with M-MuSV 124, we have suggested that high levels of p37^{mos} may be toxic to cells (15). Possibly the higher level of P85 in *ts110* MuSV-infected cells can be tolerated because the presence of the NH₂-terminal *gag* sequences in P85 or the deletion of NH₂-terminal sequences from the *v-mos* gene results in a decreased potency of the *mos* function. In contrast to p37^{mos} (15), synthesis of P85 is only slightly elevated after acute infection of NIH 3T3 cells with *ts110* MuSV. However, the absolute level of P85 after acute infection with *ts110* MuSV is similar to the level of p37^{mos} seen in cells acutely infected with M-MuSV 124 (15). Whereas P85 is the predominant *v-mos*-encoded protein detected by immunoprecipitation of *ts110* MuSV-transformed cells, it may not be the sole product of the *ts110* MuSV *v-mos* gene, since trace amounts of a smaller specifically

ts110 MuSV-infected stable cell line grown at 32°C (marked stable) was immunoprecipitated in parallel. In addition, cells acutely infected with M-MuSV 124 (marked 124) were prepared as described previously (15), labeled with [35 S]methionine, and immunoprecipitated in parallel as outlined above. All lanes shown were from the same gel. The gel was fluorographed and exposed to film for 5 days, except for the tracks shown in the right-hand "acute" A/B pair. These are the same tracks as in the left-hand acute A/B pair but exposed to film for 14 days to reveal p33 (arrow). Lanes: A, anti-C3 immunoprecipitate; B, blocked anti-C3 immunoprecipitate; M, 1 μ l of [35 S]methionine-labeled in vitro translation product from M-MuSV 124 virion RNA (14). The 37,000- and 33,000-dalton in vitro *v-mos* proteins are marked with arrows.

precipitated protein, p33, can be detected after acute infection of NIH 3T3 cells with *ts110* MuSV. Therefore, although P85 is most likely to be responsible for transformation by *ts110* MuSV, the involvement of smaller *v-mos* proteins cannot be ruled out. In this regard it is interesting that the largest *mos* protein we have detected in cells transformed by activated *c-mos* gene sequences (4) is 33,000 daltons (J. Papkoff, unpublished data).

We are indebted to Mei Lai, Chuck Van Beveren, Inder Verma, Paul Hoeprich, and Russell Doolittle for the synthetic peptides used to raise the antiserum and acknowledge Inder Verma for collaboration with the MuSV experiments. We thank Ralph Arlinghaus for providing the *ts110* MuSV-infected cells used for this study and for communicating results before publication.

This work was supported by Public Health Service grants CA14195, CA17096, and CA28458 from the National Cancer Institute. J.P. was supported by National Cancer Institute National Research Service Award CA09370.

LITERATURE CITED

- Andersson, P., M. P. Goldfarb, and R. A. Weinberg. 1979. A defined subgenomic fragment of in vitro synthesized Moloney sarcoma virus DNA can induce cell transformation upon transfection. *Cell* 16:63-75.
- Blair, D. G., M. A. Hull, and E. A. Finch. 1979. The isolation and preliminary characterization of temperature-sensitive transformation mutants of Moloney sarcoma virus. *Virology* 95:303-316.
- Blair, D. G., W. L. McClements, M. K. Oskarsson, P. J. Fischinger, and G. F. Vande Woude. 1980. Biological activity of cloned Moloney sarcoma virus DNA: terminally redundant sequences may enhance transformation efficiency. *Proc. Natl. Acad. Sci. U.S.A.* 77:3504-3508.
- Blair, D. G., M. Oskarsson, T. G. Wood, W. C. McClements, P. J. Fischinger, and G. F. Vande Woude. 1981. Activation of the transforming potential of a normal cell sequence: a molecular model for oncogenesis. *Science* 212:941-943.
- Canaani, E., K. C. Robbins, and S. A. Aaronson. 1979. The transforming gene of Moloney murine sarcoma virus. *Nature (London)* 282:378-383.
- Donoghue, D. J. 1982. Demonstration of biological activity and nucleotide sequence of an in vitro synthesized clone of the Moloney murine sarcoma virus *mos* gene. *J. Virol.* 42:538-546.
- Horn, J. P., T. G. Wood, D. G. Blair, and R. B. Arlinghaus. 1980. Partial characterization of a Moloney murine sarcoma virus 85,000-dalton polypeptide whose expression correlated with the transformed phenotype in cells infected with a temperature-sensitive mutant virus. *Virology* 105:516-525.
- Horn, J. P., T. G. Wood, E. C. Murphy, D. G. Blair, and R. B. Arlinghaus. 1981. A selective temperature-sensitive defect in viral RNA expression in cells infected with a *ts* transformation mutant of murine sarcoma virus. *Cell* 25:37-46.
- Junghans, R. P., E. C. Murphy, Jr., and R. B. Arlinghaus. 1982. Electron microscopic analysis of *ts110* Moloney murine sarcoma virus: a variant of wild-type virus with two RNAs containing large deletions. *J. Mol. Biol.* 161:229-255.
- Moloney, J. B. 1966. A virus-induced rhabdomyosarcoma of mice. *Natl. Cancer Inst. Monogr.* 22:139-142.
- Murphy, E. C., Jr., and R. B. Arlinghaus. 1982. Comparative tryptic peptide analysis of P85^{*gag-mos*} of *ts110* Moloney murine sarcoma virus and P38-P23 *mos* gene-related protein of wild-type virus. *Virology* 121:372-383.
- Papkoff, J., T. Hunter, and K. Beemon. 1980. In vitro translation of virion RNA from Moloney murine sarcoma virus. *Virology* 101:91-103.
- Papkoff, J., M. H.-T. Lai, and T. Hunter. 1982. Analysis of *v-mos* encoded proteins in cells transformed by several related murine sarcoma viruses. *J. Cell. Biochem.* 19:349-362.
- Papkoff, J., M. H.-T. Lai, T. Hunter, and I. M. Verma. 1981. Analysis of transforming gene products from Moloney murine sarcoma virus. *Cell* 27:109-119.
- Papkoff, J., I. M. Verma, and T. Hunter. 1982. Detection of a transforming gene product in cells transformed by Moloney murine sarcoma virus. *Cell* 29:417-426.
- Reddy, E. P., M. J. Smith, and S. A. Aaronson. 1981. Complete nucleotide sequence and organization of the Moloney murine sarcoma virus genome. *Science* 214:445-450.
- Reddy, E. P., M. J. Smith, E. Canaan, K. C. Robbins, S. R. Tronick, S. Zain, and S. A. Aaronson. 1980. Nucleotide sequence analysis of the transforming region and large terminal redundancies of Moloney murine sarcoma virus. *Proc. Natl. Acad. Sci. U.S.A.* 77:5234-5238.
- Sefton, B. M., T. Hunter, and W. C. Raschke. 1981. Evidence that the Abelson virus protein functions in vivo as a protein kinase which phosphorylates tyrosine. *Proc. Natl. Acad. Sci. U.S.A.* 78:1552-1556.
- Stanker, L. H., G. E. Gallick, W. S. Kloetzer, E. C. Murphy, Jr., and R. B. Arlinghaus. 1983. P85: a *gag-mos* polypeptide encoded by *ts110* Moloney murine sarcoma virus. *J. Virol.* 45:1183-1189.
- Van Beveren, C., J. A. Galleghaw, V. Jonas, A. J. M. Berns, R. F. Doolittle, D. J. Donoghue, and I. M. Verma. 1981. Nucleotide sequence and formation of the transforming gene of a mouse sarcoma virus. *Nature (London)* 289:258-262.
- Van Beveren, C., F. van Straaten, J. A. Galleghaw, and I. M. Verma. 1981. Nucleotide sequence of the genomes of a murine sarcoma virus. *Cell* 27:97-108.
- Vande Woude, G. F., M. Oskarsson, W. L. McClements, L. W. Enquist, D. G. Blair, P. J. Fischinger, J. V. Maizel, and M. Sullivan. 1979. Characterization of integrated Moloney sarcoma proviruses and flanking host sequences cloned in bacteriophage lambda. *Cold Spring Harbor Symp. Quant. Biol.* 44:735-745.
- Verma, I. M., M. H.-T. Lai, R. A. Bosselman, M. A. McKennett, H. Fan, and A. Berns. 1980. Molecular cloning of unintegrated Moloney mouse sarcoma virus DNA in bacteriophage lambda. *Proc. Natl. Acad. Sci. U.S.A.* 77:1773-1777.