Proc. Natl. Acad. Sci. USA
Vol. 78, No. 9, pp. 5593–5597, September 1981
Cell Biology

Sequence of events in the transformation process in cells infected with a temperature-sensitive transformation mutant of Moloney murine sarcoma virus

(cytoskeleton/microtubules/actin cables/cell morphology/fibroblast transformation)

RONALD L. BROWN*†, JACQUELINE PELTIER HORN‡, LINDA WIBLE§, RALPH B. ARLINGHAUS‡¶, AND B. R. BRINKLEY§

Departments of *Cell Biology and ‡Tumor Virology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030; and §Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Communicated by Theodore T. Puck, April 10, 1981

ABSTRACT **Normal rat kidney cells infected with the temperature-sensitive transformation mutant of Moloney murine sarcoma virus were used to study the biochemical and morphological changes that occur during transformation. The infected cells exhibited a normal morphology at the nonpermissive temperature (39°C) and a transformed morphology at the permissive temperature (33°C). A new viral protein was detected 2 hr after shift to the permissive temperature as a polyprotein with an estimated M_r of 85,000 (p85). Scanning electron microscopy of the cells within 5 hr after shifting them to the permissive temperature showed that they became smaller and rounded with numerous elongated microvilli. In an earlier study, changes in hexose uptake were found to occur 8–12 hr after the shift [Horn, J. P., Wood, T. G., Blair, D. G. & Arlinghaus, R. B. (1980) *Virology* 105, 516–525]. By 48 hr, the cells had the morphology of a fully transformed cell. Concomitant with the changes in the morphology were alterations in the cytoplasmic microtubule complex. At the nonpermissive temperature, the complex consisted of a lacy network of microtubules. Within 5 hr at the permissive temperature, the lacy network was still present but the microtubules were more diffusely stained and less discernible. By 48 hr, the microtubules were so diffuse that the lacy network could not be recognized. Alterations in the F-actin cables did not occur until 24 hr after shifting the cells to the permissive temperature. Enucleation of the cells at the nonpermissive temperature and shifting the cytoplasts to the permissive temperature did not result in the synthesis of detectable p85 or in any alteration of the cytoplast morphology or microtubule complex, suggesting that the temperature-sensitive lesion affects some event occurring in the nucleus.**

Cells transformed by viral or chemical carcinogens exhibit characteristics that distinguish them from their nontransformed counterparts. These characteristics include altered cell shape (1), loss of anchorage to substrate (2, 3), loss of density-dependent control of growth (4), loss of contact-inhibited mobility (5), and altered cell surface properties (6, 7). In addition, it has been shown that *in vitro* the transformed cells may display changes in their cytoskeletal system, including an altered cytoplasmic microtubule complex (CMTC) (8, 9) and the diminution of actin cables (10). The mechanism by which a transformation-specific gene product causes a wide spectrum of morphological and growth-related processes is unknown. One interesting possibility is that the transforming agent may produce a product that interacts with elements of the cytoskeleton, which then could lead to the expression of the transformed phenotype. The expression of the transformation state has been extensively studied in the avian sarcoma virus system (11). Studies involving mammalian sarcoma virus, however, have been impeded due to the lack of stable conditionally defective mutants.

Recently, Blair *et al.* (12) have isolated a stable temperature-sensitive (ts) mutant of Moloney murine sarcoma virus (Mo-MuSV), termed ts110, by using an enrichment selection procedure based on the ability of transformed cells to grow in semisolid media. Mo-MuSV was mutated by UV irradiation and used to infect normal rat kidney (NRK) cells. The ts mutants were selected by their ability to grow at 33°C but not at 39°C since those transformed cells that were able to grow in semisolid media at 39°C were killed by arabinonucleoside (cytosine arabinoside) treatment at 39°C. All clones obtained in this way are transformed at 33°C but are phenotypically normal at 39°C. One such cell clone (6m2) was characterized; it does not produce virus at either temperature but contains viral-specified polyproteins (p) of M_r 58,000 (p58) and 85,000 (p85) (13, 14). p85 is detected only at the transformed state and contains the antigenic determinants of the viral core proteins, p15, p12, and p30, in addition to other unidentified peptide sequences. These latter sequences do not appear to be antigenically related to gene products from the replication genes (*gag*, *pol*, or *env*). Therefore, it is possible that these unidentified sequences may be derived from the MuSV *src* gene. The p58 viral protein is detected at both 33°C and 39°C. It appears to be derived from the *gag* gene but lacks sequences of the viral core protein p10. Therefore, p58 is present at both the normal and the transformed state, whereas p85 is present only at the transformed state at 33°C (13, 14). In this report, we present evidence on the sequence of events correlating the appearance of p85 with the morphological and cytoskeletal alterations characteristic of the transformed state and an analysis of nuclear control of viral gene expression in the 6m2 clone.

MATERIALS AND METHODS

Cell Culture and Virus. The 6m2 clone was used (12, 13). For the temperature-shift experiments, the cells were shifted to 39°C for 48 hr and then either maintained at 39°C or shifted to 33°C.

Analysis of Viral Proteins. In some experiments, the 6m2 cells were incubated for 20 hr with [^{3}H]leucine (100 μCi/ml;

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: CMTC, cytoplasmic microtubule complex; ts, temperature sensitive; Mo-MuSV, Moloney murine sarcoma virus; NRK, normal rat kidney; p, polyprotein; P_i/NaCl, phosphate-buffered saline; Fl, fluorescein isothiocyanate conjugated; Ra-MuLV, Rauscher murine leukemia virus.

† Present address: Primate Virus Immunobiology, Biological Carcinogenesis Program, Frederick Cancer Research Center, P.O. Box B, Frederick, MD 21701.

¶ To whom reprint requests should be addressed.

1 Ci = 3.7×10^{10} becquerels) in McCoy's 5a medium/Earle's balanced salt solution containing 10% dialyzed fetal calf serum (1:1) at either 33°C or 40°C. Pulse labeling of cells, cell lysis, and immunoprecipitation were as described (13, 15, 16). Immunoprecipitates were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis on a 6–12% linear gel; radioactive bands were detected by fluorography (13).

Immunofluorescence of Microtubules. Cells were grown on 11 × 22 mm coverslips for analysis of the CMTC. The cells were fixed in 3% formaldehyde and processed for tubulin immunofluorescence according to the procedure of Brinkley *et al.* (8).

Fluorescein Isothiocyanate-Conjugated Phalloidin Staining of F Actin. Cells were grown to a 60–70% confluency on 11 × 22 mm coverslips for staining F-actin cables with fluorescein isothiocyanate-conjugated (Fl)-phalloidin, a gift from T. Wieland (Max-Planck Institut for Medical Research, Heidelberg, Federal Republic of Germany). The cells were processed by rinsing in 80 mM 1,4-piperazinediethanesulfonic acid/1 mM $MgCl_2$/1 mM EGTA, ph 6.9 (stabilizing buffer), lysed for 90 sec in stabilizing buffer/0.5% Triton X-100, and fixed in 3% formaldehyde/phosphate-buffered saline (P_i/NaCl), pH 7.3 for 30 min. Each coverslip was inverted over a drop of Fl-phalloidin (10 μg at 1 μg/ml in P_i/NaCl/1% dimethyl sulfoxide) for 1 hr. The coverslips were washed with several changes of P_i/NaCl for 1 hr and then mounted on glass slides using glycerol/P_i/NaCl, pH 8.9–9 (9:1) as mounting medium.

Cytochalasin B Enucleation. Cells were grown on 25-mm circular coverslips. For enucleation, the coverslips, cell surface down, were supported in 50–ml polyethylene centrifuge tubes by a hollow polyethylene cylinder. The cells were centrifuged in a Sorvall HB-4 swinging bucket rotor at 9000 × *g* for 60 min at 37°C in the presence of cytochalasin B (Aldrich) at 10 μg/ml in medium/10% fetal calf serum. The cytoplasts were fixed in 3% formaldehyde and processed for tubulin immunofluorescence as described for whole cells.

To examine the expression of viral proteins in cytoplasts, the cells were enucleated with cytochalasin B as described (17). Cells were grown on sterile plastic sheets at 33°C and then shifted to 39°C for 48 hr prior to enucleation. These sheets were cut from Corning tissue culture flasks and resemble microscope slides with one end rounded so as to fit the bottom of a Sorvall 50-ml centrifuge tube. Logarithmically growing cells were plated onto two plastic sheets contained in a 100-mm tissue culture dish. The cells were then incubated at 37°C for 15 min in medium/cytochalasin B (10 μg/ml). The cells were centrifuged in medium/cytochalasin B for 60 min at 12,000 × *g* in a Sorvall HB-4 swinging bucket rotor at 37°C. The plastic sheets containing the cytoplasts were placed in fresh medium and incubated for 45 min at 39°C and then shifted to 33°C. After 4 hr at 33°C, the cytoplasts were pulse labeled with [^{3}H]leucine as described for whole cells.

RESULTS

Viral Proteins. By using long-term labeling of ts110-infected NRK cells, we were able to detect the virus-specific proteins synthesized by the infected cells (Fig. 1). In these experiments, cells maintained at 33°C (*A*) or 39°C (*B*) were labeled for 20 hr with [^{3}H]leucine. The cells were then lysed in detergent-containing buffer, and the cytoplasmic extracts were challenged with the appropriate antisera: antiserum to disrupted MuSV-124 (lane 2), anti-gp69/71 (lane 3), antisera to the four core proteins, p30, p15, pp12, and p10 (lanes 4–7, respectively), anti-reverse transcriptase (lane 8), and normal goat serum (lane 9). p85 was detected only at the permissive temperature, 33°C, and was recognized by antisera to p30, p15, and p12, as well as the antiserum to total disrupted MuSV-124. p85 was not immunoprecipitated by anti-gp69/71, anti-p10, anti-reverse transcriptase, or normal goat serum. p58 was detected at both 33°C and 39°C and was recognized by the same sera that recognized p85. Thus, this experiment shows that both p85 and p58 have antigenic determinants to viral core proteins p30, p15, and p12 and that p58 is synthesized at both temperatures while p85 is synthesized only at the permissive temperature. These results support and confirm our previous studies (13, 14).

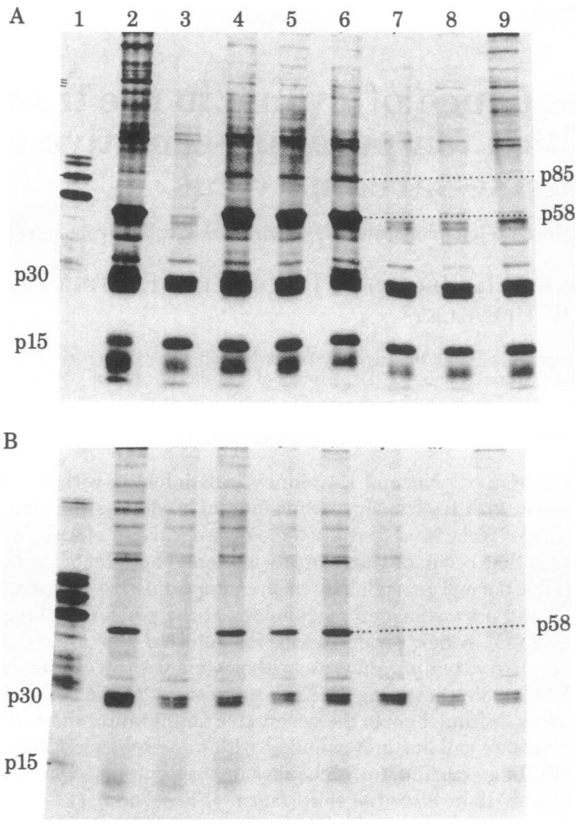

FIG. 1. Analysis of virus-specific polypeptides synthesized in ts110 MuSV-infected NRK cells at the permissive and nonpermissive temperatures. The ts110-infected NRK cells were labeled for 20 hr with [^{3}H]leucine in half-strength McCoy's medium/10% dialyzed fetal calf serum at either 33°C (*A*) or 40°C (*B*). The cells were lysed in buffer/detergent (13). Cytoplasmic extracts were prepared, divided into aliquots, and challenged. Lanes: 2, anti-MuSV-124; 3, anti-gp69/71; 4, anti-p30; 5, anti-p15; 6, anti-pp12; 7, anti-p10; 8, anti-reverse transcriptase; 9, normal goat serum. Lane 1 contains molecular weight markers (200,000–15,000) representing a 15-min pulse–chase labeling of Rauscher murine leukemia virus (Ra-MuLV)-infected NIH Swiss mouse embryo cells immunoprecipitated with anti-Ra-MuLV serum (13). Indirect precipitation was carried out by using inactivated *Staphylococcus aureus* (Cowan strain) by the method of Kessler (16). The precipitated complex was washed and suspended in a sample buffer containing $NaDodSO_4$ and 2-mercaptoethanol, and immunoprecipitates were analyzed by gel electrophoresis. Equal amounts of radioactivity were applied to each lane.

Because p85 was detected only at the permissive temperature, we wanted to relate the detection of p85 to the expression of the transformed phenotype. This was accomplished by shifting the cells maintained at the nonpermissive temperature to the permissive temperature and analyzing the viral-specific proteins synthesized at various times after the shift. Cells were pulse labeled for 15 min with [^{3}H]leucine at hourly intervals after the temperature shift (Fig. 2). Cytoplasmic extracts were then challenged with anti-pp12, and equal amounts of radioactivity were applied to each gel lane. p85 was first detectable in infected cells 2 hr after the shift to the permissive temperature. This suggests that p85 expression requires a 2-hr lag pe-

riod after the shift to the permissive temperature before synthesis can begin.

Cell Morphology. Scanning electron microscopy of the ts110 MuSV-infected cells maintained at the nonpermissive temperature showed a structure typical of nontransformed cells. The cells were flat in appearance with few microvilli (Fig. 3*a*). On shifting the cells to the permissive temperature, noticeable changes in morphology occurred within 5 hr (Fig. 3*b*). Initially, cells were smaller and slightly more rounded with discernible microvilli. After 24 hr at the permissive temperature, the cells became much smaller with numerous long microvilli (Fig. 3*c*). Finally, after 48 hr at the permissive temperature, the cells had achieved the small rounded phenotype characteristic of fully transformed cells (Fig. 3*d*). The cells remained stabilized in this phenotype thereafter.

CMTC. To further characterize the events occurring in the transformation process, the cytoskeletal system was studied at both the nonpermissive and permissive temperatures. At the nonpermissive temperature, the CMTC consisted of an extensive lacy network of long microtubules (Fig. 4*a*). Most cells displayed one prominent centrosome with microtubules radiating out toward the plasma membrane. On shifting the cells to the permissive or transforming temperature, the microtubules became shorter, less dispersed, and more diffusely stained (Fig. 4 *b–d*). This alteration was monitored 5, 24, and 48 hr after shifting to the transformed temperature. The CMTC, 5 hr after shifting to the permissive temperature, was still discernible in that the lacy network of microtubules was present (Fig. 4*b*). However, the microtubules were more diffusely stained and less discernible in comparison with the cells grown at the nonpermissive or normal temperature. At 24 hr, the microtubule network was still apparent but it was even more diffuse (Fig. 4*c*). Finally, after 48 hr, the microtubules were so diffuse that the lacy network could not be recognized in most cells (Fig. 4*d*).

F-Actin Cables. Wulf *et al.* (18) and Verderame *et al.* (19) have reported that Fl-phalloidin stains the actin cables more sharply than does actin antibody due to a decreased intracellular background fluorescence. This allows for improved resolution and reproducible differences between normal and transformed cells. Thus, we analyzed the F-actin cables in the 6m2 cells at both the permissive and nonpermissive temperatures by using Fl-phalloidin. The F-actin cables of the cells at the nonpermissive temperature were easily discernible as long thick brightly

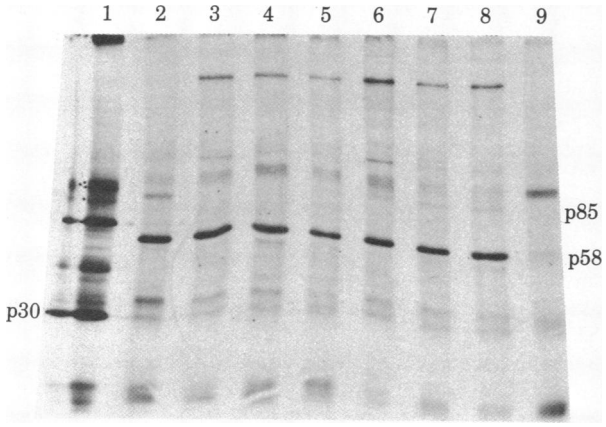

FIG. 2. Synthesis of viral-specific polypeptides in ts110 MuSV-infected NRK cells after a temperature shift. Seven identical flasks of ts110-infected NRK cells were maintained at 40°C for 48 hr prior to the shift; one flask was maintained at 33°C. Cells grown at 40°C were then shifted to 33°C for the indicated times before a 15-min pulse labeling with [^{3}H]leucine, and cytoplasmic extracts were challenged. Lanes: 2, cells maintained at 33°C; 3, cells maintained at 40°C; 4–8, cells maintained at 40°C and then shifted to 33°C at 1, 2, 3, 4, and 5 hr, respectively (after shifting, all challenged with anti-pp12); 9, cells maintained at 40°C and challenged with normal goat serum. Lane 1 represents pulse–chase labeling of Ra-MuLV-infected cells with [^{3}H]-leucine followed by immunoprecipitation with anti-Ra-MuLV serum.

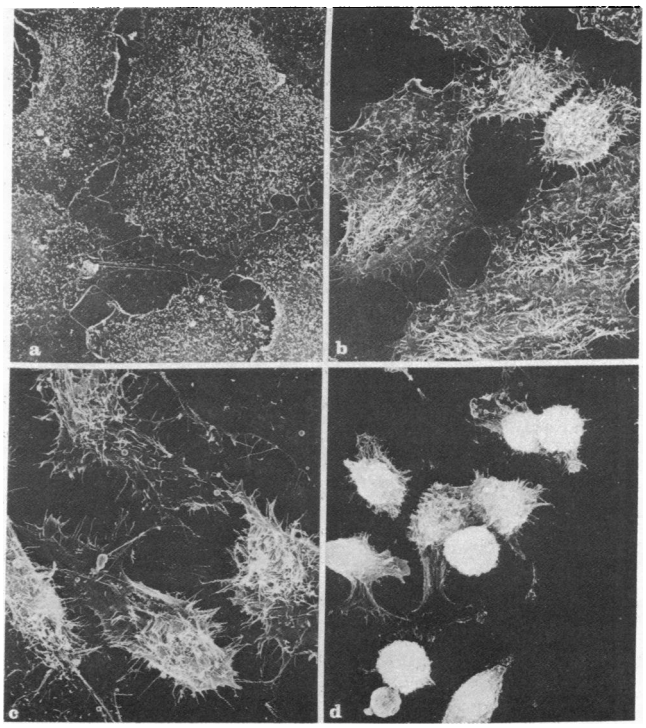

FIG. 3. Morphology of ts110 MuSV-infected NRK cells after a temperature shift. (*a*) Cells maintained at 39°C. (×380.) (*b–d*) Cells 5, 24, and 48 hr, respectively, after a shift to 33°C. (*b* and *d*, ×470; *c*, ×700.)

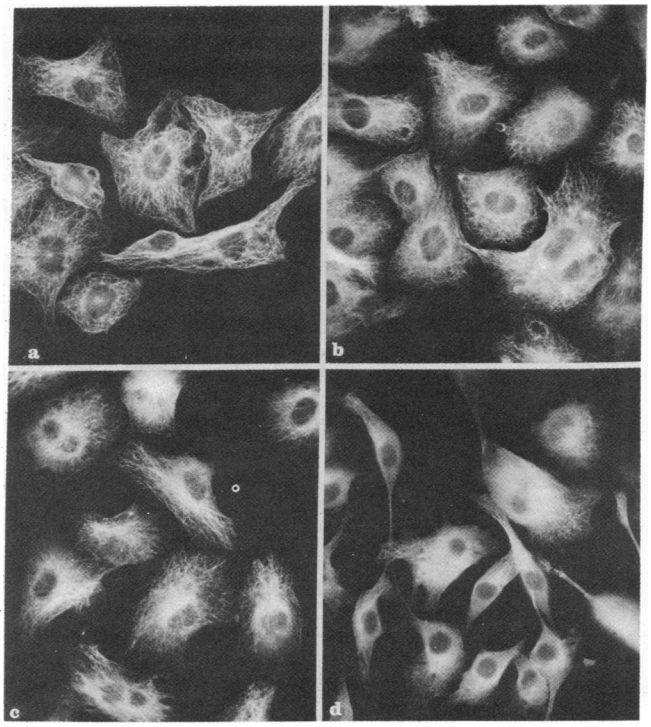

FIG. 4. Immunofluorescence of the CMTC of ts110 MuSV-infected NRK cells after a temperature shift. (*a*) Cells maintained at 39°C. (×260.) (*b–d*) Cells 5, 24, and 48 hr, respectively, after a shift to 33°C. (×240.)

fluorescing cables (Fig. 5*a*). No alterations were noticeable in the F-actin cables 5 hr after a shift to the permissive temperature (Fig. 5*b*). However, 24 hr after shifting, the F-actin cables were reduced in number and shorter and less well defined than at the nonpermissive temperature (Fig. 5*c*). Finally, after 48 hr at the permissive temperature, most cells had few discernible F-actin cables, which is characteristic of transformed cells (19).

Nuclear–Cytoplasmic Control of Gene Expression for Transformation. To ascertain whether or not the ts lesion affected some step in the nucleus or in the cytoplasm, the 6m2 cells were enucleated with cytochalasin B. Cytoplasts from the 6m2 cells cultured at the nonpermissive temperature showed a CMTC characteristic of nucleated cells maintained at the same temperature (Fig. 6*a*). Shifting the cytoplasts to the permissive temperature for 20 or 44 hr resulted in no apparent alteration of the CMTC (Fig. 6 *b* and *c*). To determine whether cells maintained at 39°C and then enucleated and shifted to 33°C synthesize p85, such cytoplasts were shifted to 33°C for 4 hr and then pulse labeled for 15 min with [^{3}H]leucine. The detergent-treated extract was immunoprecipitated with anti-p12. Analysis by NaDodSO$_4$/polyacrylamide gel electrophoresis showed p58 but not p85 (Fig. 7), whereas nonenucleated cells shifted to 33°C did synthesize p85. This result implies that functional mRNA for p85 is not made and not exported to the cytoplasm at 39°C in the mutant virus-infected cells. As a control, a partially enucleated cell population (<30% enucleation) was treated as described above; both p58 and p85 were detected (results not shown). This experiment suggests that the ts lesion concerns some nuclear event and that this nuclear event must take place in order to (*i*) synthesize p85 and (*ii*) convert the cell to a transformed phenotype. It is tempting to conclude that the synthesis of p85 is necessary for the cellular alterations observed in the transformed culture, although other interpretations are possible.

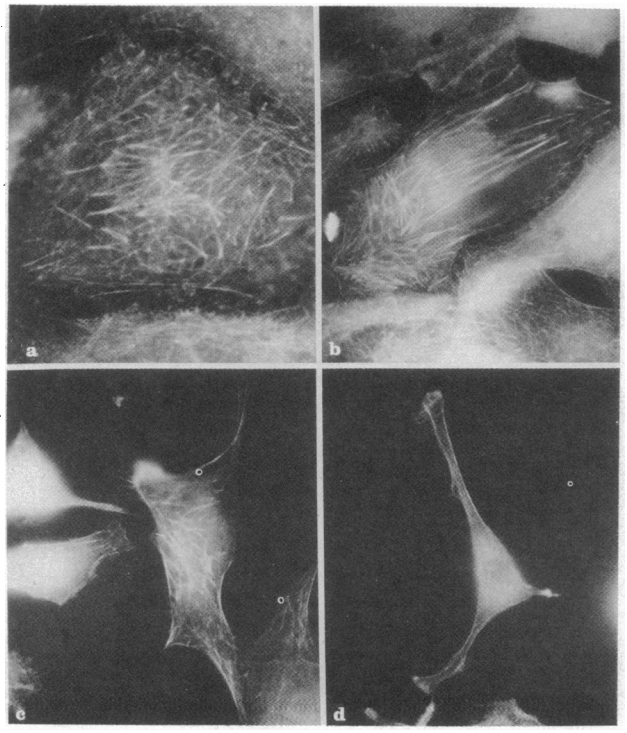

FIG. 5. Fl-phalloidin stained F-actin cables of ts110 MuSV-infected NRK cells after a temperature shift. (*a*) Cells maintained at 39°C. (×450.) (*b–d*) Cells 5, 24, and 48 hr, respectively, after a shift to 33°C. (*b* and *c*, ×440; *d*, ×450.)

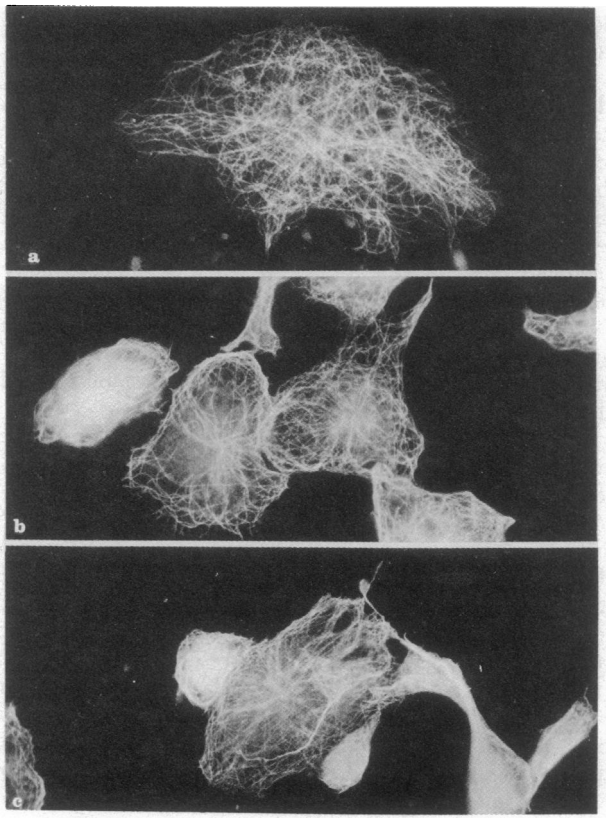

FIG. 6. CMTC of ts110 MuSV-infected NRK cytoplasts. (*a*) Cytoplasts at the nonpermissive temperature. (×320.) (*b* and *c*) Cytoplasts after 20 and 40 hr, respectively, at the permissive temperature. (*b*, ×530; *c*, ×340.)

DISCUSSION

The sequence of events occurring in the transformation of mammalian cells has been difficult to delineate. However, the recent discovery of a stable ts transformation mutant of Mo-MuSV affords the opportunity to follow both biochemical and morphological aspects of the transformation process in more detail (12). We have found that NRK cells infected with the ts Mo-MuSV, ts110, produce proteins of M_r 58,000 (p58) and 85,000 (p85). Viral p58 is produced at both the permissive and nonpermissive temperatures, whereas p85 is produced only at the permissive temperature (13, 14).

The alterations that occur with transformation appear to be complete in the infected cells 48 hr after shifting to the permissive temperature. The sequence of events occurring in the transformation process appears to be as follows. When infected cultures were shifted from 39°C to 33°C, Mo-MuSV-specified p85 was detectable after a lag of 2 to 3 hr. Changes in the cell morphology concomitant with alterations in the expression of the CMTC occurred within 5 hr after shifting ts110-infected NRK cells to the permissive temperature. A detectable change in the rate of hexose uptake was found to occur 8–12 hr after the shift (14). The F-actin cables in the cell did not appear to be altered until after 24 hr at the permissive temperature.

The results suggest that the synthesis of p85 that occurs 2 or 3 hr after a shift to the permissive temperature preceeds the alterations in cell morphology, CMTC, and rate of hexose uptake. Thus, these cellular changes are among the initial or at least very early events in the transformation process, in contrast to the changes in F-actin cables, which appear to occur later. Whether alterations in the CMTC cause the morphology of the cell to change or vice versa is difficult to ascertain.

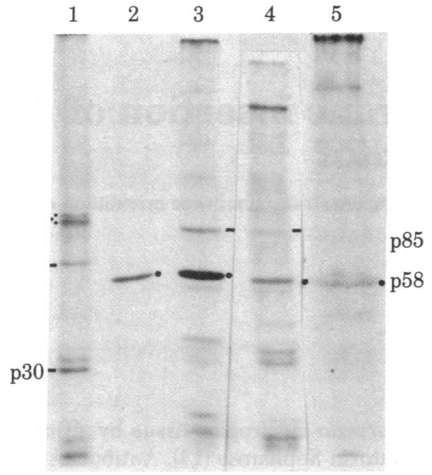

FIG. 7. Analysis of virus-specific polypeptides synthesized in enucleated ts110 MuSV-infected NRK cells first maintained at 39°C and then shifted to 33°C. Cells were labeled for 15 min with [^{3}H]leucine at 33°C and 39°C, and the cytoplasmic extracts were challenged by antisera to pp12. Lanes: 1, Pulse chase of Ra-MuLV-infected cells immunoprecipitated with anti-Ra-MuLV; 2, cells maintained at 39°C for 48 hr and then pulse labeled; 3, cells maintained at 33°C; 4, 5-hr control derived from cells maintained at 39°C for 48 hr and then shifted to 33°C for 5 hr and pulse labeled; 5, cells were maintained at 39°C for 48 hr and enucleated at 37°C with cytochalasin B (requiring about 1 hr of manipulation) and the resulting cytoplasts were incubated for 4 hr at 33°C and then pulse labeled.

Recently Sen and Todaro (20) reported that a low molecular weight protein phosphokinase from m3MuSV pseudotype virions binds to actin and microtubule proteins. In the latter, it decreased the rate and extent of *in vitro* polymerization. Also, similar results were found for ts110 MuSV particles, which have a thermolabile actin-binding protein kinase (21), and Burr *et al.* (22) have reported that the *src* gene product of Rous sarcoma virus associates with cytoskeletons extracted from chicken embryo fibroblast. These reports on isolated cytoskeletal elements can be interpreted to mean that the *src* gene product does indeed interact with the cytoskeleton.

By using electron microscopic immunochemistry, Willingham *et al.* (23) have localized the *src* protein (p21) in cells transformed by the Harvey strain of MuSV to the plasma membrane. Therefore, whether the Harvey *src* gene product effects the CMTC or plasma membrane, which subsequently alters the other, needs to be studied further. The data presented here suggest that the initial events in the transformation process are the alterations in cell morphology and CMTC followed later by diminution of F-actin cables. This supports the hypothesis that the microtubule–microfilament system in cells may be involved in the transformation process (24–26).

A ts lesion involved in transformation could affect either a transcriptional or a translational event. Beug *et al.* (27) reported that ts68 mutants of Rous sarcoma virus were able to express a transformed phenotype in the absence of a nucleus, thus suggesting that the ts lesion in this virus is independent of the nucleus and resides in the *src* gene product itself. ts110 MuSV-infected cells maintained at nonpermissive temperatures and then enucleated were unable to express p85 and any morphological or CMTC alterations after they were shifted to the permissive temperature. This result indicates that the lesion in ts110 MuSV resides in the nucleus, possibly involving some reaction concerned with the production of active p85 cytoplasmic mRNA. Inhibition of RNA synthesis with actinomycin D prior to shifting the cells to the permissive temperature blocks the subsequent synthesis of p85 and the morphological alterations associated with transformation, further suggesting a nuclear lesion in ts110 MuSV (28).

Knowledge of the sequence of events necessary to give the pleiotrophic effects of transformation will increase our understanding of the biological complexity of this process. It would also allow us to detect the primary site of action of the *src* gene product. This information could be useful in the early detection of malignant transformation.

We thank James Syrewicz and Donna Turner for their technical assistance and Becky Bazer and Linda Lawyer for their assistance in manuscript preparation. This research was supported in part by grants from the Robert A. Welch Foundation (G-429) and the National Cancer Institute (CA-25465, CA-16672, and CA-2260) and by contract NO1-CO-75380 with the National Cancer Institute. It is a joint research project of the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, and the John S. Dunn Research Foundation of Houston, Texas. J.P.H. is a Predoctoral Fellow supported by the Rosalie B. Hite Foundation.

1. Temin, H. M. & Rubin, H. (1958) *Virology* **6**, 669–688.
2. MacPherson, I. & Montagnier, L. (1964) *Virology* **23**, 291–294.
3. Risser, R. & Pollack, R. (1974) *Virology* **59**, 477–489.
4. Todaro, G. J., Green, H. & Goldberg, B. D. (1964) *Proc. Natl. Acad. Sci. USA* **51**, 66–73.
5. Gail, M. H. & Boone, C. W. (1971) *Exp. Cell Res.* **68**, 226–288.
6. Burger, M. M. (1973) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 91–101.
7. Pollack, R. E. & Burger, M. M. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 1074–1076.
8. Brinkley, B. R., Fuller, B. M. & Highfield, D. P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4981–4985.
9. Fuller, G. M. & Brinkley, B. R. (1976) *J. Supramol. Struct.* **5**, 497–514.
10. Pollack, R., Osborn, M. & Weber, K. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 994–998.
11. Bishop, J. M. (1978) *Annu. Rev. Biochem.* **47**, 35–88.
12. Blair, D. G., Hull, M. A. & Finch, E. A. (1929) *Virology* **95**, 303–316.
13. Horn, J. P., Wood, T. G., Blair, D. G. & Arlinghaus, R. B. (1980) *Virology* **105**, 516–525.
14. Wood, T. G., Horn, J. P., Robey, W. G., Blair, D. G. & Arlinghaus, R. B. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 747–754.
15. Naso, R. B., Arcement, L. J. & Arlinghaus, R. B. (1975) *Cell* **4**, 31–36.
16. Kessler, S. W. (1975) *J. Immunol.* **115**, 1617–1624.
17. Lucas, J. J., Szekely, E. & Kates, J. R. (1976) *Cell* **7**, 115–122.
18. Wulf, E., Deboben, A., Bautz, F. A., Faulstich, H. & Wieland, T. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4498–4502.
19. Verderame, M., Alcorta, D., Egnor, M., Smith, K. & Pollack, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6624–6628.
20. Sen, A. & Todaro, G. J. (1979) *Cell* **17**, 347–356.
21. Sen, A., Todaro, G. J., Blair, D. G. & Robey, W. G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3617–3621.
22. Burr, J. G., Dreyfuss, G., Penman, S. & Buchanan, J. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3484–3488.
23. Willingham, M. C., Pastan, I., Shih, T. Y. & Scolnick, E. M. (1980) *Cell* **19**, 1005–1014.
24. Puck, T. T., Waldren, C. A. & Hsie, A. W. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1943–1947.
25. Puck, T. T. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4491–4495.
26. Edleman, G. M. & Yahara, I. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2047–2051.
27. Beug, H., Claviez, M., Jockusch, B. M. & Graf, T. (1978) *Cell* **14**, 843–856.
28. Horn, J. P., Wood, T. G., Murphy, E. C., Jr., Blair, D. G. & Arlinghaus, R. B. (1981) *Cell* **25**, 37–46.