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# Simian Virus 40 and Polyoma Virus Induce Synthesis of Heat Shock Proteins in Permissive Cells

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During the lytic infection of monkey and mouse cells with simian virus 40 and polyoma virus, respectively, the preferentially increased synthesis of two host proteins of 92,000 and 72,000 $M_r$ was observed by 15 to 20 h after infection besides the general stimulation of most cellular proteins. The incubation of uninfected monkey and mouse cell cultures for 30 to 60 min at 43.5°C induced the enhanced synthesis of at least three proteins of 92,000, 72,000 and 70,000 $M_r$, the last one being the major heat shock protein of mammalian cells. Two-dimensional gel electrophoresis and partial proteolytic digestion confirmed that the same 92,000- and 72,000-$M_r$ proteins are stimulated by virus infection and thermal treatment. In simian virus 40-infected CV-1 cells, we also observed the weak stimulation of a 70,000-$M_r$ protein comigrating in gel electrophoresis with the major heat shock protein. The 92,000-, 72,000-, and 70,000-$M_r$ proteins of monkey cells are structurally very similar to the corresponding proteins of mouse cells. In immunoprecipitations, no specific association of these proteins to simian virus 40 T antigens was noticed.

In a variety of organisms and cell cultures, from bacteria and yeasts to humans, a mild heat shock was found to induce the vigorous synthesis of a few characteristic proteins. In *Drosophila melanogaster*, where this phenomenon has been extensively investigated, the induction of the heat shock proteins is paralleled by a strong reduction in the synthesis of most proteins made before the heat shock. In avian and mammalian cells, decreased protein synthesis is less evident, but the increased synthesis of at least two proteins in the range of 85,000 to 95,000 $M_r$ and 70,000 to 75,000 $M_r$ was observed; these proteins may be related to two of the major heat shock proteins in insects. The increased synthesis of the same or similar proteins has been reported for cells subjected to a variety of treatments, such as exposure to amino acid analogs, chelating drugs, heavy metal ions, arsenite, and other sulfhydryl reagents (for a review, see reference 19).

The lytic infection of mouse and monkey cell cultures with polyoma virus or simian virus 40 (SV40), respectively, induces an increased synthesis of the majority of cellular proteins (9). Among these, two strongly stimulated host proteins fall into the same size range as the proteins mentioned above (E. W. Khandjian, P. Arrigo, T. Rose, and J.-M. Matter, Experientia **36**:749, 1980). Here, we show that the two proteins that are stimulated by virus infection are also induced by thermal treatment of uninfected mouse and monkey cells.

## MATERIALS AND METHODS

**Cell cultures, virus infections, and thermal treatment.** Confluent primary mouse kidney cultures and confluent cultures of CV-1 (African green monkey) cells in 9-cm-diameter petri dishes were mock infected or infected with polyoma virus or SV40, respectively, and incubated at 37°C as described previously (9). For thermal treatment (heat shock), cultures were incubated at 43.5°C for 1 h before labeling at 37°C for 2 h with 100 μCi of [$^{35}$S]methionine (500 to 1,000 Ci/mmol; The Radiochemical Centre, Amersham, United Kingdom) in 2 ml of methionine-free medium.

**Extraction of proteins.** After [$^{35}$S]methionine labeling, the cell monolayers were washed with cold phosphate-buffered saline, total proteins were solubilized by the addition of sodium dodecyl sulfate (SDS) sample buffer (68 mM Tris-hydrochloride [pH 6.8], 2% SDS, 2% 2-mercaptoethanol, 0.01% bromophenol blue, 15% glycerol) (10), and the viscous extract was sonicated. Alternatively, cultures put on ice were lysed with 0.5% Nonidet P-40 in 0.1 M Tris-hydrochloride (pH 9)–0.1 M NaCl–5 mM $MgCl_2$ for 10 min, and the lysates were centrifuged at 30,000 × $g$ for 30 min at 4°C. To the supernatant 0.5 volume of threefold-concentrated SDS sample buffer was added, and the samples were heated in a boiling water bath for 1.5 min. For two-dimensional gel electrophoresis, cells were lysed in urea lysis buffer (9.5 M urea, 2% Nonidet P-40, 5% 2-mercaptoethanol, 2% ampholytes [LKB-Produkter AB, Bromma, Sweden]) (13). Insoluble

material was removed by centrifugation at 12,000 × *g* for 10 min.

**Gel electrophoresis.** Proteins were separated by SDS-polyacrylamide gel electrophoresis (10) in vertical slab gels. Apparent molecular weights ($M_r$) were determined by the use of the following markers: β-galactosidase, 116,000; phosphorylase *a*, 97,000; transferrin, 77,000; bovine serum albumin, 68,000; glutamate dehydrogenase, 41,000; carbonic anhydrase, 29,000; and myoglobin, 17,000. After electrophoresis, gels were fixed and stained with 0.5% Coomassie brilliant blue in 20% (wt/vol) trichloroacetic acid, destained, dried, and exposed to Kodak XR-5X-Omat R films for 1 to 5 days. For quantitation, autoradiographs were scanned with a microdensitometer, and peak areas were determined by a Hewlett-Packard (model 3385) computer; in addition, radioactivity was counted in excised bands. Two-dimensional gel electrophoresis was done by the method of O'Farrell (13) with minor modifications: for electrofocusing, the cathodic electrolyte was 0.2 M NaOH, the anodic electrolyte was 0.1 M $H_3PO_4$, and 0.01% bromophenol blue was included in the overlay solution to follow migration. Electrofocusing was for 5,000 V h. After SDS-polyacrylamide gel electrophoresis in the second dimension, $^{35}$S-labeled proteins were revealed by fluorography (11).

**Partial proteolytic digestion.** Preparative polyacrylamide gels (7.5% acrylamide) were briefly rinsed in water and dried. Protein bands revealed by autoradiographv were excised, rehydrated, put into sample wells of a 15% SDS-polyacrylamide gel, and overlaid with 25 μl of buffer containing 10% glycerol, 0.01% bromophenol blue, and 0.5 or 0.05 μg of *Staphylococcus aureus* V8 protease (Miles Laboratories, Inc.) (2). Peptide bands were revealed by fluorography (11).

**Immunoprecipitation.** One-milliliter samples of cell extracts obtained with 0.5% Nonidet P-40 (pH 9) were reacted with 20 μl of hamster anti-SV40 tumor serum or with 10 μl of a mouse serum directed against spontaneously transformed mouse cells (T-AL/N; kindly provided by T. Rose). Immune complexes were isolated by immunoaffinity chromatography with protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) (20).

## RESULTS

**Cellular proteins induced by virus infection and by thermal treatment.** During the lytic and abortive infection of mouse and monkey cells with polyoma virus and SV40, an increased synthesis of most cellular proteins was observed (9). Among these, two proteins with apparent $M_r$ of about 92,000 and 72,000 were particularly stimulated. An increased synthesis of these two proteins was detected by labeling with [$^{35}$S]methionine by 15 to 20 h and by protein staining by about 24 h after infection and took place also when cellular and viral DNA replication was inhibited with 5-fluorodeoxyuridine or 1-β-D-arabinofuranosylcytidine (Khandjian et al., Experientia **36:**749, 1980; and unpublished data).

To test for a possible relation of the two virus-induced proteins with mammalian heat shock proteins, we analyzed the virus-induced and thermally induced proteins of CV-1 and mouse kidney cells on the same SDS-polyacrylamide gel (Fig. 1). Virus-infected monkey and mouse cell cultures showed an increased synthesis of the 92,000-$M_r$ protein (resolved as a doublet)and of the 72,000-$M_r$ protein. The stimulation of the two proteins was detected both by protein staining (Fig. 1, lanes 2 and 5) and by autoradiography (Fig. 1, lanes 2′ and 5′). The proteins with about 46,000 $M_r$ observed in the same lanes are the major viral capsid protein VP1 of SV40 and polyoma virus.

The thermal treatment of CV-1 and mouse kidney cells led to an increased labeling of the 92,000- and 72,000-$M_r$ proteins and the appearance of a new, strongly labeled protein with an apparent $M_r$ of 70,000 (Fig. 1, lanes 3, 3′, 6, and 6′). In uninfected and polyoma-infected mouse kidney cells kept at 37°C, this protein was not detected (Fig. 1, lanes 4, 5, 4′, and 5′), but in the corresponding CV-1 cells, the 70,000-$M_r$ protein was visible as a faint band (Fig. 1, lanes 1, 2, 1′, and 2′). In CV-1 cells but not in mouse kidney cells, two other proteins with apparent $M_r$ of about 115,000 and 65,000 showed increased labeling after thermal treatment (Fig. 1, lane 3′). Figure 2 shows the microdensitometer scans of the autoradiographs from Fig. 1. Labeled proteins from virus-infected (Fig. 2A and C) and thermally treated cultures (Fig. 2B and D) are compared with those from uninfected cultures kept at 37°C. The quantitative analysis of the scans is given in Table 1. The stimulation of $^{35}$S incorporation in total monkey and mouse proteins by virus infection was 1.5- and 1.7-fold, respectively. In SV40-infected CV-1 cells, the increase in labeling of the 92,000- and 72,000-$M_r$ proteins was significantly higher than that of the total proteins. In polyoma-infected mouse kidney cells, preferential labeling of the 92,000- and 72,000-$M_r$ proteins was less pronounced. It should be noted that the labeling of total and individual proteins in virus-infected cultures varies with the time of infection (9). The labeling of total and individual proteins after thermal treatment was closely similar in monkey and mouse cells (Table 1), the stimulation of the 92,000- and 72,000-$M_r$ proteins being in the same order of magnitude as in virus-infected cells.

To determine more specifically how the 92,000-, 72,000-, and 70,000-$M_r$ proteins respond to virus infection and thermal treatment, we labeled parallel cultures of mock-infected and SV40-infected CV-1 cells which were either maintained at 37°C or subjected to thermal treatment. The proteins were electrophoresed on a high-resolution SDS-polyacrylamide gel. Comparison of the different lanes in Fig. 3 yielded the

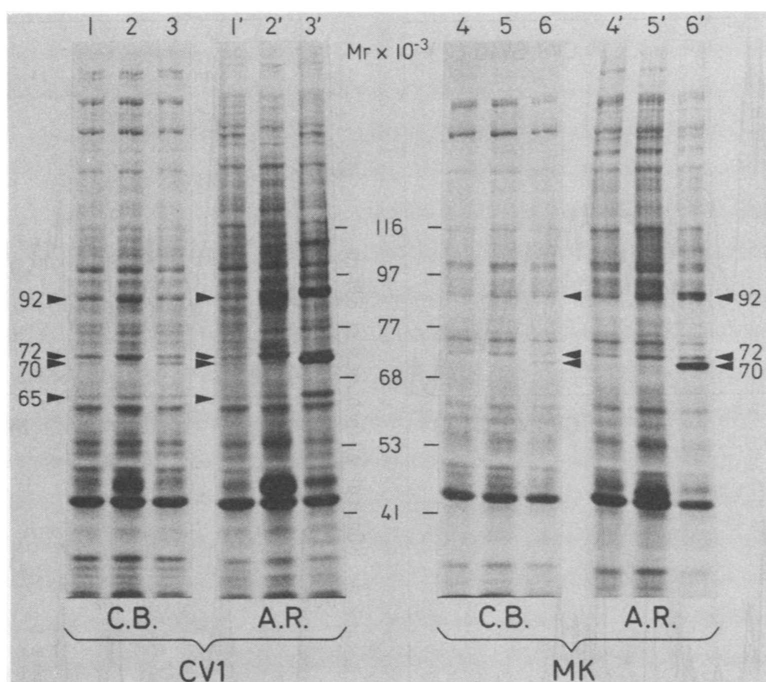


FIG. 1. Virus-induced and thermally induced monkey and mouse cell proteins. All cultures were labeled at 37°C with [$^{35}$S]methionine from 27 to 29 h after infection or mock-infection. Proteins were extracted with 0.5% Nonidet P-40 (pH 9) and analyzed by SDS-polyacrylamide gel electrophoresis (7.5% acrylamide). Lanes 1 to 3, Mock-infected, SV40-infected, and thermally treated mock-infected CV-1 cells, respectively; lanes 4 to 6, mock-infected, polyoma-infected, and thermally treated mock-infected mouse kidney (MK) cells; lanes 1 to 6, Coomassie blue (C.B.)-stained gel; lanes 1' to 6', corresponding autoradiography (A.R.). Arrows indicate the major virus-induced and heat shock-induced proteins of 92,000, 72,000, 70,000 and 65,000 $M_r$.

following results: (i) increased synthesis of the 92,000-$M_r$ protein was observed under both conditions (Fig. 3, lanes 2, 2', 3', and 4'); (ii) the 72,000-$M_r$ protein was strongly stimulated by virus infection (Fig. 3, lanes 2, 2', 4, and 4') and moderately by thermal treatment (Fig. 3, lane 3'); (iii) the major heat shock protein of 70,000 $M_r$ (Fig. 3, lanes 3' and 4') showed weak stimulation by virus infection (Fig. 3, lanes 2 and 2'). Thus, both the 72,000-$M_r$ and the 70,000-$M_r$ proteins responded, although differently, to virus infection and thermal treatment.

**Comparison of virus-induced and heat shock-induced cell proteins. (i) Two-dimensional gel electrophoresis.** Fluorographs of two-dimensional gel electrophoresis of proteins extracted from mock-infected, SV40-infected, and thermally treated CV-1 cultures are reproduced in Fig. 4. Among the numerous host proteins showing increased synthesis after virus infection, the 92,000-, 72,000-, and 70,000-$M_r$ proteins were easily recognized. Comparison of the proteins from virus-infected cells with those from thermally treated cells showed that the 92,000 (pI 5.2), the 72,000 (pI 5.8), and the 70,000 (pI 6.1) proteins migrated to the same positions, thus indicating that indeed the same proteins were stimulated by virus infection and by thermal treatment. However, the thermally induced 92,000- and 70,000-$M_r$ proteins showed some charge heterogeneity which was not observed after virus infection. Since no inhibitors of DNA replication had been used, the synthesis of viral capsid proteins VP1 with the known charge heterogeneity (9, 14) and VP2 (17, 18) was observed. SV40-infected cultures also showed strong stimulation of a protein with about 53,000 $M_r$ (pI 5.1), which we did not characterize further. The labeling of actin ($M_r$, 43,000; pI 5.35) showed no significant changes in the three differently treated cultures.

**(ii) Partial proteolysis.** The major virus-induced and heat shock-induced proteins of monkey and mouse cells were further characterized by partial proteolytic digestion with staphylococcal protease V8. Figure 5A shows the digests of the 92,000-$M_r$ proteins present in mock-infected mouse kidney and CV-1 cells (Fig. 5, lanes 3 and III, respectively), in virus-infected cells (Fig. 5, lanes 2 and II), and in heat shock-

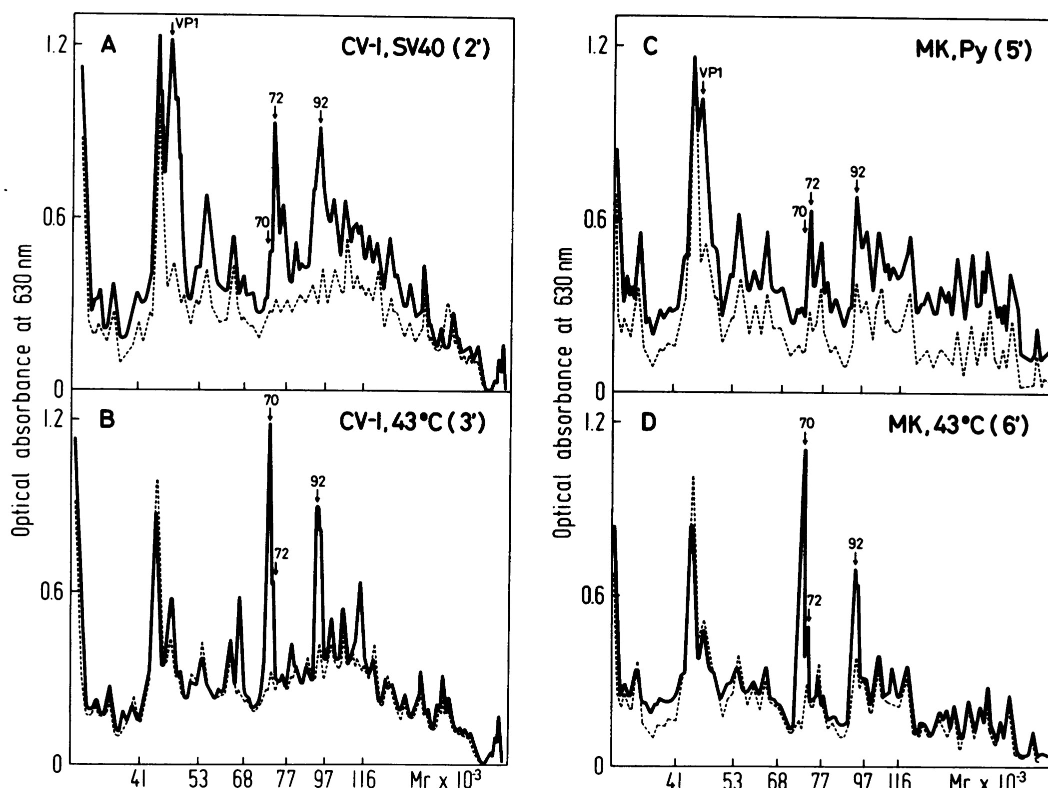


FIG. 2. Densitometer tracings of the autoradiographs shown in Fig. 1. Virus-infected (A and C) and thermally treated (B and D) cultures (solid line, tracing of lanes 2′, 3′, 5′, and 6′, respectively, in Fig. 1) are compared with mock-infected cultures kept at 37°C (dashed line, tracings of lanes 1′ and 4′ in Fig. 1). (A and B) CV-1 cells; (C and D) mouse kidney cells.

treated cells (Fig. 5, lanes 1 and I). These analyses showed that the same 92,000-$M_r$ protein already present in mock-infected cultures was induced by virus infection and by thermal treatment, both in mouse and monkey cells. In addition, the similarity of the patterns obtained for the mouse protein and the monkey protein was striking; we detected only very minor differences between the two, which are indicated in the figure by dashes.

TABLE 1. Stimulation of protein synthesis by virus infection and thermal treatment

| Protein | CV-1 | | Mouse kidney | |
|---|---|---|---|---|
| | Inf/mock[a] | Hs/mock[a] | Inf/mock | Hs/mock |
| Total proteins | 1.5 | 1.1 | 1.7 | 1.1 |
| 92,000 | 2.8 | 2.1 | 1.8 | 2.3 |
| 72,000 | 4.1 | 2.1 | 2.0 | 1.7 |
| 70,000 | —[b] | 6.5 | —[c] | >6[c] |
| Actin | 1.1 | 1.0 | 1.2 | 1.1 |

[a] Figures give the peak area of individual proteins or the area of total proteins from virus-infected (Inf) or thermally treated (Hs) cultures over those from uninfected cultures kept at 37°C (mock).

[b] The quantitation of the 70,000-$M_r$ protein in virus-infected CV-1 cells was not possible due to the overlap with the 72,000-$M_r$ band.

[c] The 70,000-$M_r$ protein was not detected in infected and uninfected mouse kidney cultures kept at 37°C; the value for its stimulation by thermal treatment was estimated in comparison with CV-1 cultures.

Figure 5B shows the analysis of the virus-induced and thermally induced 72,000-, 70,000-, and 65,000-$M_r$ proteins. In the preparative gels, the separation of the 72,000- and 70,000-$M_r$ proteins of thermally treated CV-1 cells was not sufficient to excise the two bands separately. Figure 5, lanes 5 and V, shows the partial digests of the mixture of the 72,000- and 70,000-$M_r$ proteins from thermally treated mouse kidney and CV-1 cells, respectively, whereas lane 4 shows the pattern of the 70,000-$M_r$ heat shock protein of mouse kidney cells. Since the labeling of the 70,000-$M_r$ protein was three- to fivefold higher than that of the 72,000-$M_r$ protein, most bands observed in lanes 5 and V have to be attributed to the 70,000-$M_r$ protein. This is also concluded from a comparison of lane 4 (mouse, 70,000) and lane 5 (mouse, 70,000 and 72,000). The patterns obtained in lanes 4, 5, and V again show a striking similarity, indicating a close

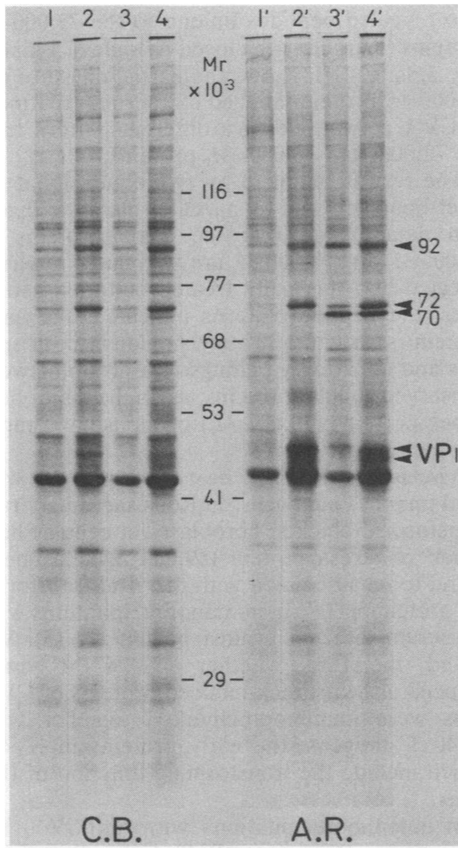


FIG. 3. Virus-induced and thermally induced proteins of CV-1 cells. All cultures were labeled at 37°C with [$^{35}$S]methionine from 44 to 46 h after infection or mock infection. Proteins were extracted with 0.5% Nonidet P-40 (pH 9) and analyzed by high-resolution SDS-polyacrylamide gel electrophoresis (10% acrylamide; 210- by 140- by 0.7-mm gel). Lanes 1 and 2, Mock-infected and SV40-infected CV-1 cells, respectively; lanes 3 and 4, thermally treated mock-infected and SV40-infected CV-1 cells, respectively. Lanes 1 to 4, Stained gel (C.B.); lanes 1' to 4', corresponding autoradiography (A.R.). VP1, Major viral capsid proteins ($M_r$, 46,000).

structural relation between the 70,000-$M_r$ heat shock proteins of mouse and monkey cells; the characteristic bands for this protein are indicated by open triangles. Figure 5, lanes 6 and VI, show the digests of the virus-induced 72,000-$M_r$ proteins of mouse and monkey cells, respectively, and lane 7 shows that of the 72,000-$M_r$ protein present in uninfected mouse kidney cells. We were unable to isolate a sufficiently labeled and clean band for the 72,000-$M_r$ protein of mock-infected CV-1 cells. The patterns of the mouse and monkey 72,000-$M_r$ proteins were again closely similar. These digests showed a few bands which might correspond to bands observed with the 70,000-$M_r$ proteins, but they

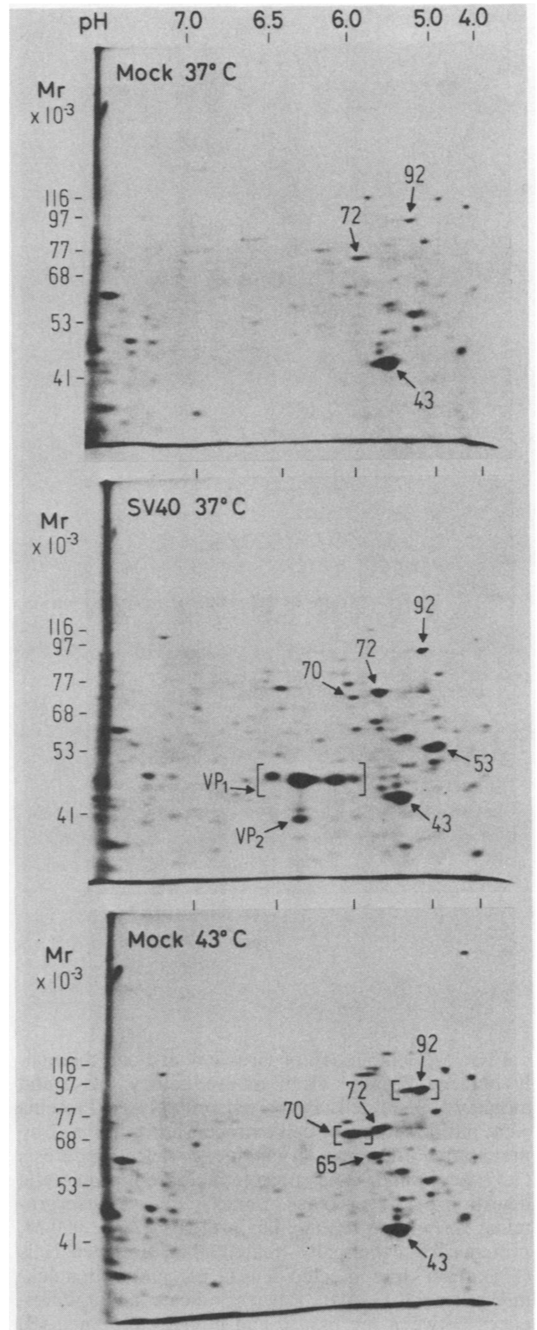


FIG. 4. Two-dimensional gel electrophoresis of $^{35}$S-labeled proteins of mock-infected, SV40-infected, and thermally treated, mock-infected CV-1 cells. All cultures were labeled at 37°C with [$^{35}$S]methionine from 44 to 46 h after infection or mock infection. Thermal treatment (Mock 43°C) was done at 43.5°C for 1 h before labeling. Proteins were extracted with urea lysis buffer. First dimension, Isoelectric focusing for 5,000 V h; second dimension, SDS-polyacrylamide gel electrophoresis (7.5% acrylamide). Numbers indicate the major virus- and thermally induced proteins. VP, Viral capsid proteins; 43, actin.

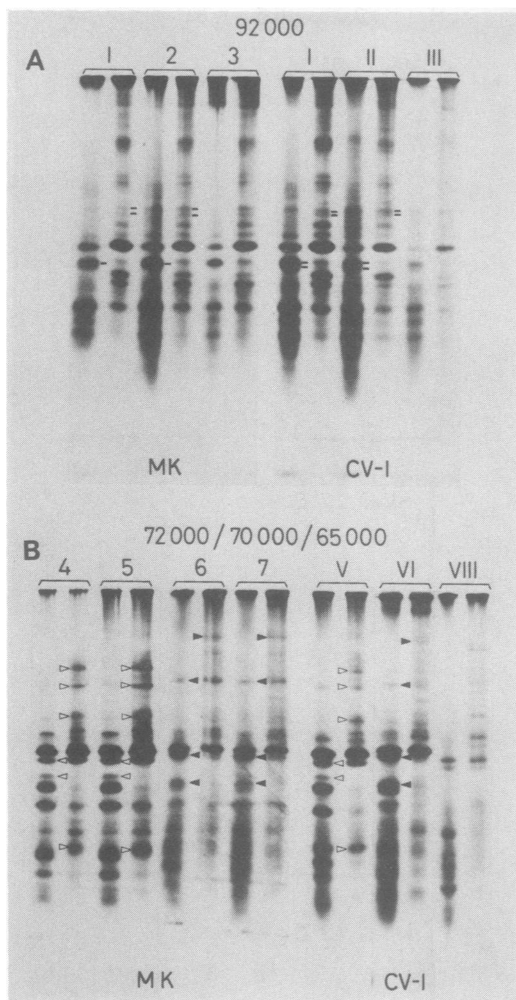


FIG. 5. Comparison of virus-induced and thermally induced proteins from mouse kidney (MK) and monkey (CV-1) cells by partial proteolysis. Proteins were purified from the cell extracts shown in Fig. 2 by preparative SDS-polyacrylamide gel electrophoresis (7.5% acrylamide) and subjected to partial proteolytic digestion by V8 protease. Lanes 1 to 7, Mouse proteins; lanes I to VIII, monkey proteins. (A) 92,000-$M_r$ proteins from thermally treated, mock-infected cells (1, I), from virus-infected cells (2, II), and from mock-infected cells (3, III). Dashes indicate minor differences between the mouse and monkey proteins. (B) 70,000-$M_r$ protein (lane 4) and mixture of 70,000- and 72,000-$M_r$ proteins (lanes 5 and V) from thermally treated mock-infected cells; 72,000-$M_r$ proteins from virus-infected (lanes 6 and VI) and from mock-infected (lane 7) cells; 65,000-$M_r$ protein from thermally treated mock-infected cells (lane VIII). Open triangles indicate unique peptides of the 70,000-$M_r$ major heat shock proteins; closed triangles indicate unique peptides of the 72,000-$M_r$ proteins. In each series of digests, the left lane was digested with 0.5 μg of V8 protease per sample, and the right lane was digested with 0.05 μg.

also revealed peptides unique to the 72,000-$M_r$ proteins (marked with closed triangles). Finally, Fig. 5, lane VIII, shows the partial digest of the 65,000-$M_r$ protein induced by thermal treatment in CV-1 cells, yielding a different pattern than the 72,000- and 70,000- $M_r$ proteins.

The results obtained by two-dimensional gel electrophoresis and by partial proteolytic digestion demonstrate that the same proteins of 92,000 $M_r$ and 72,000 $M_r$ are stimulated in virus-infected and thermally treated cultures. Moreover, the peptide patterns indicated that each protein is structurally very similar in both species and apparently belongs to a group of well-conserved cellular proteins, as do the major heat shock proteins (70,000 $M_r$) of mouse and monkey cells.

**Association of cellular heat shock proteins with viral tumor (T) antigens.** In Rous sarcoma virus-transformed chicken fibroblasts, a cellular heat shock or stress protein (89,000 $M_r$) has been found to be associated with the viral transforming protein pp60$^{src}$ in immunoprecipitations with antiserum directed against pp60$^{src}$ (1, 15). We tested, therefore, whether any of the virus-induced or heat shock-induced proteins of CV-1 cells were immunoprecipitated together with SV40 T antigens, the early proteins of SV40, which include the transforming function of this virus.

In immunoprecipitations with anti-SV40 tumor serum (Fig. 6a), SV40 large T antigen (88,000 $M_r$) was easily detected by staining (Fig. 6, lanes 2 and 4) and by autoradiography (Fig. 6, lanes 2′ and 4′) in extracts from infected cells. In the same lanes, a faint band corresponding to the virus-induced 72,000-$M_r$ protein was also observed. Immunoprecipitates from thermally treated mock-infected and SV40-infected cultures showed by autoradiography an additional band corresponding to the 70,000-$M_r$ heat shock protein (Fig. 6, lanes 3′ and 4′, respectively). However, bands of the 72,000- and 70,000-$M_r$ proteins with similar intensity were also observed in immunoprecipitates of corresponding cell extracts, using a mouse antiserum containing no antibodies against SV40 T antigens (Fig. 6b). This and the fact that radioactivity was very low in these bands compared with radioactivity present in these proteins in the cell extracts suggested that there was no significant association of these proteins with SV40 T antigens. Their presence in the immunoprecipitates was rather due to unspecific binding to the antiserum or to protein A-Sepharose, as was also observed for other proteins, e.g., actin (43,000 $M_r$) and viral capsid protein VP1 (46,000 $M_r$). The 92,000-$M_r$ protein is expected to migrate slightly slower than SV40 large T antigen, making its detection difficult. However, the sharpness of

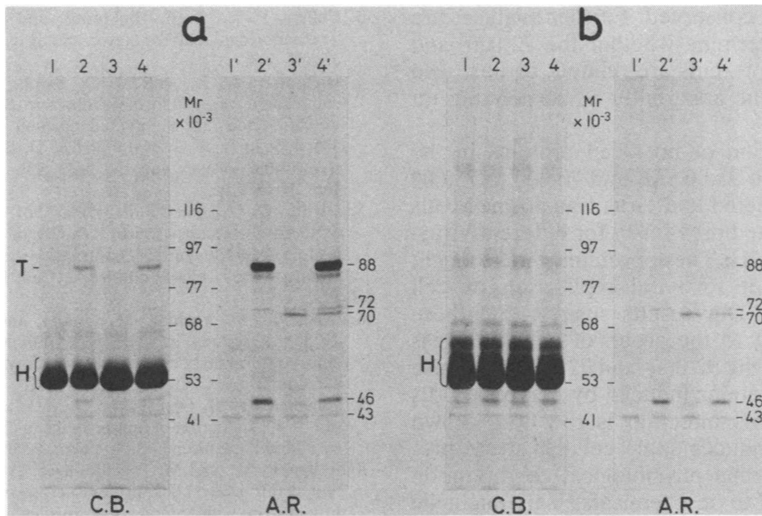


FIG. 6. Immunoprecipitations of SV40 T antigen and heat shock proteins from extracts of untreated and thermally treated CV-1 cells. All cultures were labeled with [$^{35}$S]methionine from 24 to 25 h after infection. Immunoprecipitated proteins were analyzed by SDS-polyacrylamide gel electrophoresis (7.5% acrylamide). (a) Immunoprecipitations with hamster anti-SV40 tumor serum; (b) immunoprecipitations with a mouse serum directed against spontaneously transformed mouse cells (T-AL/N). Lanes 1 and 2, Mock-infected and SV40-infected cells maintained at 37°C, respectively; lanes 3 and 4, thermally treated mock-infected and SV40-infected cells, respectively. C.B., Coomassie blue staining (lanes 1 to 4); A.R., corresponding autoradiographs, exposed for 4 weeks (lanes 1' to 4'). T, SV40 large T antigen (88,000 $M_r$); H, immunoglobulin G heavy chains. Numbers refer to $M_r$ ($\times 10^3$) of marker proteins, virus-induced and heat shock-induced proteins (72 and 70), viral capsid protein (46), and actin (43).

the stained and autoradiographed large T antigen bands (Fig. 6a, lanes 2, 4, 2', and 4', respectively) also seems to rule out a significant association of the 92,000-$M_r$ monkey protein with SV40 T antigens. Immunoprecipitations with cell extracts made in the presence of 0.1% SDS at pH 7.0 (1) and with extracts from SV40-infected mouse kidney cells led to the same conclusions (data not shown). Since the role of SV40 T antigens in the initiation and maintenance of transformation (21) is different from that of pp60$^{src}$, our results should not be understood as a contradiction of those reports.

## DISCUSSION

The lytic infection of mouse cells with polyoma virus and of monkey cells with SV40 strongly enhanced the synthesis of two cellular proteins (92,000 and 72,000 $M_r$). The same proteins were also stimulated by the thermal treatment of uninfected cells and, therefore, they belong to the group of mammalian heat shock or stress proteins (19). At present, the biological functions of these proteins in a cell responding to various stress situations are not known. The virus-induced and thermally induced 92,000-$M_r$ proteins, which formed a double band in gel electrophoresis, had the same isoelectric point and yielded the same pattern upon partial proteolytic digestion. The monkey and mouse 92,000-$M_r$ proteins were structurally very similar and correspond most likely to the 89,000-$M_r$ chicken protein, which is strongly stimulated by thermal treatment and by arsenite (1, 7, 15). This conclusion is based on the comparison of tryptic peptide analyses of the 89,000-$M_r$ chicken protein (1, 15) and of the mouse and monkey 92,000-$M_r$ proteins (unpublished data). Recently, considerable structural conservation of the larger heat shock protein ($M_r$, 84,000 to 92,000) from *Drosophila*, chickens, and humans has been found by tryptic peptide analyses (R. Voellmy, personal communication) and by immunological cross-reactions (8). The other protein which was stimulated by virus infection (72,000 $M_r$) also comigrated in two-dimensional gel electrophoresis with a protein induced in uninfected cells by thermal treatment. The major heat shock protein of mouse and monkey cells had an apparent $M_r$ of 70,000 and thus corresponds to the main stress protein of other mammalian cells (19). The synthesis of the 70,000-$M_r$ protein was moderately stimulated in SV40-infected CV-1 cells, and the virus-induced protein comigrated in two-dimensional gel electrophoresis with the major heat shock protein of CV-1 cells. Partial proteolytic digestion showed that both the 72,000- and the 70,000-$M_r$ proteins of mouse and monkey

cells are highly conserved. Further analyses are required to determine whether the 72,000- and the 70,000-$M_r$ proteins are related, as has been suggested for the apparently corresponding rat proteins (5).

The stimulation of host cell proteins in the ranges 89,000 to 95,000 $M_r$ and 70,000 to 75,000 $M_r$ in virus-infected and virus-transformed cells has been reported previously for different viruses (3, 4, 6, 16, 21). These proteins were thought to be important for viral replication or cell transformation. Our results suggest that these proteins belong to the group of cellular stress proteins, as do the 92,000- and 72,000-$M_r$ mouse and monkey proteins induced by polyoma virus and SV40. Virus infection is the first known stimulus that induces some cellular stress proteins under normal physiological conditions in the absence of toxic chemicals. This might be the reason why some differences between virus-induced and thermally induced proteins were noticed. It should also be pointed out that the increased synthesis of heat shock proteins could be observed after the exposure of cultures to 43.5°C for 10 min only; in virus-infected cells, the induction of these proteins occurred by 15 to 20 h after infection, corresponding to the asynchronous transition from early to late phase of lytic infection when cells enter S-phase. Since the increased synthesis of heat shock proteins is only one among many changes observed in infected cells, we are unable to speculate on their possible role. Recently, it has been reported that adenovirus induces the synthesis of the 70,000-$M_r$ heat shock protein during the lytic infection of HeLa cells and that this induction is controlled by an early viral gene (12). It remains to be shown whether these findings with virus-infected cell cultures reflect processes occurring in organisms which respond with higher temperature (fever) to viral infections.

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