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Semliki Forest Virus Capsid Protein Acts as a Pleiotropic Regulator of Host Cellular Protein Synthesis

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The Semliki Forest virus capsid (C) protein was introduced into various target cells by electroporation-, liposome-, and erythrocyte-ghost-mediated delivery. Data are presented which show that the incorporated C protein is biologically active and, at low concentrations (10^3 to 10^4 molecules per cell), markedly induces host cellular protein synthesis (average value, up to 90%). On the other hand, high concentrations (10^5 to 10^6 molecules per cell) led to a significant inhibition (average value, up to 60%). The cellular response to C protein was found to be identical in P3X63Ag8 suspension cells, CV-1 cells, and GpBind4 cells. Following electroporation-mediated delivery of C-protein molecules, both induction and repression of cellular protein synthesis were immediate, whereas with liposome-mediated delivery these events were delayed by about 1 h. Maximum stimulation and repression occurred between 0 and 1 h after delivery of C protein and decreased thereafter to reach control values at about 4 h. The analysis of the proteins synthesized suggests that low amounts of microinjected C protein are responsible for the induction of classes with specific M_rs, whereas high amounts lead to an inhibition of overall protein synthesis.

Semliki Forest virus (SFV), one of the best-characterized RNA viruses, belongs to the family *Togaviridae*, which includes many pathogenic members. SFV consists of an icosahedral nucleocapsid that is composed of the capsid (C) protein and a single-stranded genomic 49S RNA with a positive polarity. The nucleocapsid itself is surrounded by a lipid envelope studded with glycoprotein spikes which are essential for virus attachment and penetration into host cells. The nucleocapsid contains 180 copies of a single type of C protein (8). This protein has an M_r of about 33,000 and consists of 267 amino acid residues with a blocked N terminus (2).

The multiple interactions of parental as well as progeny C-protein molecules of SFV and Sindbis virus with cellular and viral components are only partially understood. The following functions have been attributed to the C protein: (i) the C proteins of SFV and Sindbis virus contain autoproteolytic activity (1, 11, 17); (ii) their highly purified nucleocapsids exhibit phosphokinase activity (27; M. R. Michel and H. Koblet, unpublished data); (iii) the nucleocapsid protein interacts transiently with binding sites on the large ribosomal subunit during core assembly and disassembly (28, 29, 31); (iv) C-protein molecules interact with the virus-specified polymerase complex, and it has been proposed that this interaction leads to a negative feedback mechanism in the synthesis of the subgenomic 26S mRNA (3); (v) finally, data from in vitro experiments (30) suggest that the C protein is the component responsible for the shutoff of host protein synthesis in SFV-infected cells. These observations taken together lend support to the hypothesis that SFV C-protein molecules may act in the infected cell in a fashion similar to a pleiotropic regulator protein.

It is difficult to study these presumed pleiotropic effects in a regularly infected cell, since many of the different virus-controlled processes such as transcription, translation, and core assembly appear to be intimately connected and cannot be studied separately. Therefore, we investigated some of the interactions of the C protein in the intact cell. Highly purified C-protein molecules were introduced into recipient cells by electroporation-, liposome-, and erythrocyte-ghost-mediated delivery. This approach offers the advantage over an in vitro system that the cells can be used as a living test tube, in which the presumed pleiotropic interactions of the delivered C-protein molecules can be studied in detail under cytoplasmic conditions.

MATERIALS AND METHODS

Buffers. TNE consisted of 5 mM Tris hydrochloride (pH 7.4), 145 mM NaCl, and 0.1 mM EDTA. TN consisted of 20 mM triethanolamine (pH 7.4) and 100 mM NaCl. TNEM was made up of 20 mM triethanolamine (pH 7.4), 100 mM NaCl, 1 mM EDTA, and 0.05% 2-mercaptoethanol. Lysis buffer consisted of 25 mM Tris hydrochloride (pH 7.4), 137 mM NaCl, 5 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.6 mM Na_2HPO_4, and 0.5% sodium dodecyl sulfate (SDS). TUA was made up of 0.5% Triton X-100 (TX-100), 8 M urea, and 5% acetic acid (pH 4.2). OUA was made up of 1% *n*-octyl-β-D-glucopyranoside (octylglucoside), 8 M urea, and 5% acetic acid (pH 4.2).

Cells. The mouse myeloma suspension cell line P3X63Ag8, CV-1, Vero, and GpBind4 cells were grown in reinforced Dulbecco modified Eagle medium (DMEM) containing 10% fetal bovine serum.

Virus. SFV was propagated in BHK-21 cells as described previously (12).

Isolation of C protein by cation-exchange chromatography. Isolation of C protein by cation-exchange chromatography was performed by a modified procedure of the method described by Omar and Koblet (21). After the C protein was isolated in the presence of the nonionic detergent TX-100 by a first chromatographic step through a sulfopropyltrisacryl column, TX-100 was exchanged by octylglucoside as follows. The C protein was subjected to rechromatography by reloading a 1-ml sulfopropyltrisacryl column in the presence

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of TUA. The column was then washed consecutively with 10 ml of 320 mM NaCl in TUA, 10 ml of 320 mM NaCl in TUA in which the TX-100 concentration was 0.05%, and 20 ml of 320 mM NaCl in OUA. The C protein was eluted with 70% recovery in a linear NaCl gradient in OUA consisting of 2.5 ml of 320 mM NaCl and 2.5 ml of 750 mM NaCl. The C protein was then dialyzed against decreasing molarities of urea and acetic acid (6 M and 3%, 3 M and 2%, and 1.5 M and 1.5%, respectively) and finally against 1% acetic acid. The protein was then lyophilized, suspended in water, and subjected to a second lyophilization procedure. The protein was kept in water at a concentration ranging between 2 and 5 mg/ml and stored at −70°C.

^{125}I labeling of C protein. Highly purified C protein was iodinated by a modified ^{125}I-labeled enzymobead method (14). By SDS-polyacrylamide gel electrophoresis (PAGE), the iodinated C protein had an apparent M_r of 33,000.

Preparation of the C-protein carrier. (i) Encapsulation of C protein into liposomes. Liposome preparation (using 10 μmol of a mixture consisting of oleate-phosphatidylethanolamine at a molar ratio of 3:7), encapsulation of the C protein (suspended in TNE buffer), and extrusion through polycarbonate membranes (final pore diameter, 0.2 μm) were performed as described previously (24, 25). Unilamellar liposomes were then separated from free C protein by column chromatography through Sepharose 4B, with TNE used as the elution buffer. Before use, the fractions containing the liposomes were diluted sixfold with phosphate-buffered saline (PBS).

(ii) Encapsulation of C protein into erythrocyte ghosts. We found that the most efficient loading and resealing procedure was a combination of the methods described by Kaltoft and Celis (13) and Sugawa et al. (26).

Delivery of C protein. (i) Electroporation. Electroporation, diffusion loading, and resealing of the P3X63Ag8 suspension cells and radiolabeling of these cells with [^{35}S]methionine were performed as described previously (18). For morphological studies, the P3X63Ag8 cells into which C protein was delivered were centrifuged onto gelatin-coated cover slips and flooded with cold 4% formaldehyde in PBS. After fixation and permeabilization with 0.1% TX-100 C protein was located with rabbit antibodies directed against C protein, and then with rhodamine isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (IgG) F(ab)$_2$, followed by rhodamine isothiocyanate-conjugated rabbit anti-goat IgG F(ab')$_2$ (Bio-Science Products, Emmenbrücke, Switzerland). Microscopy was performed with a fluorescence microscope (Nikon) by using 35 mm film (TMY 400-ASA; Eastman Kodak Co., Rochester, N.Y.).

(ii) Liposome-mediated delivery. Monolayer cultures in costar plates (six wells each, 9.6 cm^2 per well containing about 1.5 × 10^6 CV-1 cells) were incubated for 30 min at 37°C with 0.2 ml of liposomes. Thereafter, the cells were washed with serum-free DMEM and incubated for 5 min in the presence of 21% (vol/vol) glycerol in PBS. After removal of the glycerol the cells were washed twice with serum-free DMEM and incubated in DMEM containing 10% fetal bovine serum until they were radiolabeled with [^{35}S]methionine. The uptake of C protein following liposome delivery was assessed as follows. After delivery the cells were trypsinized (trypsin and EDTA at 0.25 and 0.02%, respectively) for 5 min at 37°C, and the reaction was stopped by the addition of DMEM containing 10% fetal bovine serum. The cells were then centrifuged in a 0.4-ml Eppendorf tube through a cushion of 1 M NaCl in PBS (pH 5) layered on top of Abil AV 200 (T. Goldschmidt AG, Essen, Federal Republic of Germany). The radioactivity present in the cell pellet and the supernatant was determined as described previously (18).

(iii) Erythrocyte-ghost-mediated delivery. As target cells were used the 3T3 cell line GpBind4 (a generous gift of J. Sambrook, University of Texas, Dallas), which constitutively expresses the hemagglutinin of influenza virus on its surface (23). The trypsin treatment and pH-triggered fusion of the erythrocyte ghosts containing C-protein molecules were performed as described by Doxsey et al. (6).

To determine the intracellular amount of C protein in those deliveries in which nonradiolabeled molecules were transferred, ^{125}I-labeled C protein was added as a tracer.

Incorporation studies of liposome-delivered C protein into progeny virus. Confluent Vero cells (about 3 × 10^6 cells) were infected with SFV at a multiplicity of infection of about 100 PFU per cell. The cells were exposed for 5 h postinfection to small (200 nm), unilamellar liposomes in which ^{125}I-labeled C protein was encapsulated. The progeny virus was collected at 20 h postinfection, and the cellular debris was removed by differential centrifugation (9,000 × *g*, 30 min, 4°C; A8.24 Kontron rotor). Polyethylene glycol 6000 (110 mg/ml), NaCl (32 mg/ml), and sucrose (40 mg/ml) were then added to the supernatant, which was stirred in the cold for 30 min and left thereafter for 12 h at 4°C. The virus was then pelleted (6,000 × *g*, 30 min, 4°C; A8.24 rotor), suspended in PBS, layered onto a 20 to 60% discontinuous sucrose gradient in PBS, and sedimented at 82,000 × *g* at 4°C for 2 h in a rotor (SW27.1). The fractions containing the virus band were pooled and pelleted by centrifugation. The pelleted virus was suspended in TNEM buffer containing 1% 3-(3-cholamidopropyl-dimethylammonio)propanesulfonate (CHAPS) and left at room temperature for 30 min. Thereafter, the virus was overlayed onto a preformed 10 to 30% sucrose gradient in TN buffer containing a layer of 60% sucrose and sedimented at 145,000 × *g* at 4°C for 2.5 h in a rotor (SW41). Fractions were collected from above and analyzed for optical densities at 260 nm, for radioactivity in a gamma counter, and by SDS-PAGE (details see below).

[^{35}S]methionine incorporation into protein. Cellular protein synthesis was determined as described previously (18). Briefly, the electroporated P3X63Ag8 suspension cells were labeled for 30 min with 30 μCi of [^{35}S]methionine (1,120 Ci/mM; Dupont, NEN Research Products, Boston, Mass.) in 100 μl of medium (5% serum) which contained only 1/15th the regular unlabeled methionine concentration. The cells were pelleted, suspended in 0.5 ml of DMEM, washed by recentrifugation, and lysed in lysis buffer. The CV-1 cells delivered with liposomes and the GpBind4 cells exposed to erythrocyte ghosts were each labeled with 0.4 ml of the medium containing [^{35}S]methionine described above.

The protein concentrations of the cellular extracts were determined as described by Lowry et al. (16).

SDS-PAGE analyses. To analyze the proteins of induced, repressed, and control cells, SDS lysates of equal protein concentrations (11 μg of protein per sample) were subjected to SDS-PAGE (10 to 15% acrylamide gradient in the separating gel and 5% acrylamide in the stacking gel). ^{14}C-labeled molecular weight markers consisted of lysozyme (M_r, 14,400), soybean trypsin inhibitor (M_r, 21,500), carbonic anhydrase (M_r, 31,000), ovalbumin (M_r, 45,000), bovine serum albumin (M_r, 66,200), phosphorylase *b* (M_r, 92,500), and β-galactosidase (M_r, 116,250), which were all purchased from Bio-Rad Laboratories (Richmond, Calif.). The gels were treated with 1 M sodium salicylate, dried, and exposed to X-ray film as described previously (4). The autoradio-

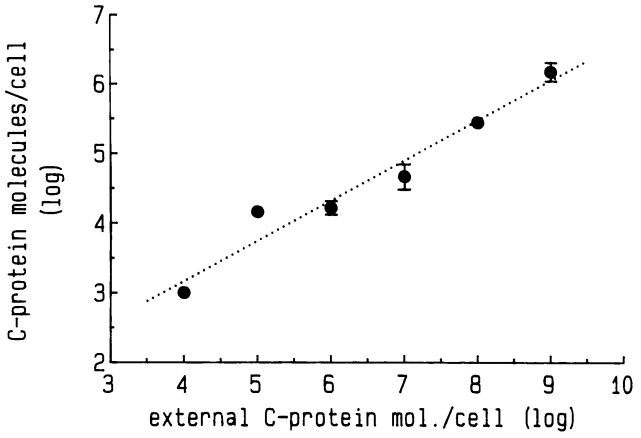

FIG. 1. Electroporation-mediated uptake of C protein. P3X63Ag8 suspension cells (10^5 cells per assay) were electroporated in the presence of increasing amounts of ^{125}I-labeled C protein (*x* axis), and the uptake into cells (*y* axis) was determined as described previously (18). Nonelectroporated control cells showed that the cell surface-attached C protein never exceeded more than 5% (18).

graphs were then scanned with a video densitometer (model 620; Bio-Rad).

RESULTS

Transfer of C-protein molecules into target cells by various delivery techniques. Purified C-protein molecules were transferred into various target cells such as P3X63Ag8 suspension cells, CV-1 cells, Vero cells, and GpBind4 cells. By using liposome- and erythrocyte-ghost-mediated delivery, the amount of C protein incorporated per cell was regulated by varying its concentration during encapsulation. For electroporation-mediated delivery, the uptake of C protein was linearly dependent on its extracellular concentration over a range of about 5 log units (Fig. 1). Saturation was not achieved with 10^9 C-protein molecules per cell (corresponding to about 10^6 incorporated molecules per cell). Figure 2 demonstrates that following electroporation C-protein molecules entered directly into the cytosolic compartment of P3X63Ag8 cells. When they were resealed, the cells were fixed and the C protein was subsequently visualized with antibodies directed against C protein, followed by fluorescent second and third antibodies. About 95% of the electroporated cells exhibited fluorescence. Figure 2a shows that the bulk of the fluorescence was diffuse, and it was evenly distributed throughout the cell and did not merely adhere to the cell surface. In addition, the absence of dotted fluorescence ruled out the possibility that the uptake of C protein was due to pinocytosis only. By comparison, nonelectroporated control cells which were exposed to C protein showed negligible fluorescence (Fig. 2c). Identical results were obtained when cells were loaded with rhodamine-labeled C protein.

Participation of liposome-delivered C protein in progeny virus formation. To study any presumptive interaction of transferred C-protein molecules with cellular constituents, it was mandatory to prove that this protein retained its biological activity following purification. To this end ^{125}I-labeled C protein (about 10^6 molecules per cell) was delivered via liposomes into Vero cells which were infected with SFV 5 h before. The cells showed a normal cytopathic effect; and the harvested and purified virus revealed, in a discontinuous sucrose gradient, that a significant amount (a calculated average of about 0.25%, i.e., approximately one ^{125}I-labeled molecule per two nucleocapsids) of the C protein present in progeny virus consisted of liposome-delivered molecules. To reveal whether the ^{125}I-labeled C protein was indeed confined to the nucleocapsid and not merely associated with the envelope proteins, the sucrose gradient-purified virus was treated with the zwitterionic detergent CHAPS. Figure 3 shows the sedimentation pattern of the CHAPS-treated virus in a linear 10 to 30% sucrose gradient which was formed on a 60% sucrose layer. Under these conditions roughly 50% of the treated virus sedimented in the gradient as an envelope-free nucleocapsid, whereas the remainder was collected as virus in the 60% sucrose layer. The inset of Fig. 3 also shows that the ^{125}I label that cosedimented with the virions (lane 3) and nucleocapsids (lane 4) migrated as an intact C-protein monomer on SDS-PAGE. These results strongly suggest that a fraction of the liposome-delivered C protein participated in the morphogenesis of nucleocapsids and the maturation of progeny virus, and thus retained its biological activity.

Induction and inhibition of protein synthesis depend on the concentration of delivered C-protein molecules. Following electroporation-mediated delivery, protein synthesis was immediately assessed by incubating the target cells in the presence of [^{35}S]methionine for 30 min at 37°C, whereas with liposome- and erythrocyte-ghost-mediated delivery, radiolabeling was delayed by 1 h. Figure 4 shows that low concentrations of incorporated C-protein molecules had a profound effect on the protein synthesis of the target cell. With all three delivery techniques that were applied, protein synthesis was induced significantly and did not depend on the cell type. Maximum stimulation was observed with about 10^3 C-protein molecules incorporated per cell (average increase, 90 ± 34%), whereas 10^4 molecules per cell showed a reduced stimulation (average increase, 59 ± 13%). Incorporation of about 10^2 molecules per cell resulted in a slight stimulation which, however, never exceeded 10% (average increase, 9 ± 4.3%). On the other hand, in the presence of high concentrations of C protein (10^5 and 10^6 molecules per cell), a significant inhibition of protein synthesis was noticed (average inhibitions, 39 ± 13% and 60 ± 17%, respectively). The large deviation in induction and repression shown in Fig. 4 is explained by the fact that the amount of C protein incorporated per cell varied by about ±50% between experiments. Control experiments in which bovine serum albumin (M_r, 66,000) was delivered revealed that in the range of 10^2 to 10^6 molecules incorporated per cell, protein synthesis was neither significantly increased nor decreased (Fig. 4D), and the protein patterns were comparable to those of nondelivered control cells (data not shown). These results further strengthen the notion that C-protein-triggered stimulation and repression of protein synthesis are specific events.

Time course of induction of protein synthesis. Figure 5 depicts the time course of induction of protein synthesis observed in two different cell lines following electroporation- and liposome-mediated delivery of low amounts of C protein. The data show that maximum stimulation was an immediate event in electroporated cells; i.e., it took place after the P3X63Ag8 suspension cells were resealed (10 min at 37°C) (18) and incubated in the presence of [^{35}S]methionine. When protein synthesis of the electroporated cells was assessed after various time periods following resealing, induction of protein synthesis decreased progressively to reach control values at 4 h after electroporation. Figure 5 also shows that the time course in the case of liposome-mediated delivery of C-protein in CV-1 cells was different.

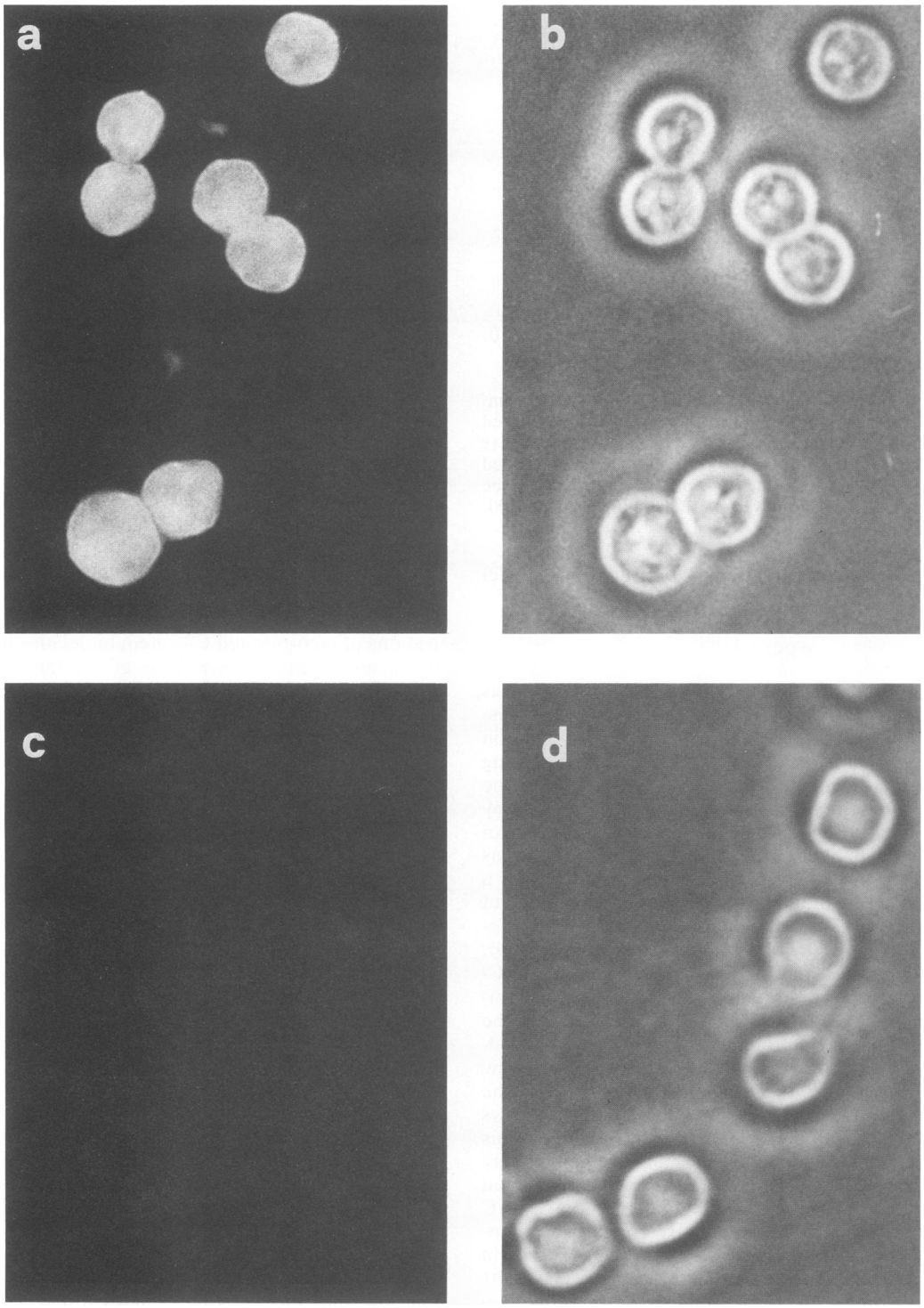

FIG. 2. Immunological detection of electroporation-mediated delivery of C protein. P3X63Ag8 cells were electroporated in the presence of C protein (about 10^6 molecules incorporated per cell). After diffusion loading (10 min at 0°C) and resealing (10 min at 37°C), the cells were fixed and then stained with rabbit anti-C-protein antibodies followed by rhodamine-tagged goat anti-rabbit and rabbit anti-goat antibodies (a). C-protein-exposed, nonelectroporated control cells were fixed and stained as described above (c). (b and d) Identical fields, as in a and c, respectively, photographed with phase-contrast optics.

Following a 30-min incubation step of the liposome-encapsulated C protein with the target cells, protein synthesis was monitored by 30-min pulses with [^{35}S]methionine at the times indicated in Fig. 5. The result shows that there was a lag of approximately 1 h before maximum stimulation of protein synthesis occurred. Thereafter, similar to electroporation-mediated C-protein delivery, induction regressed to reach control values 4 h after liposome exposure. When the

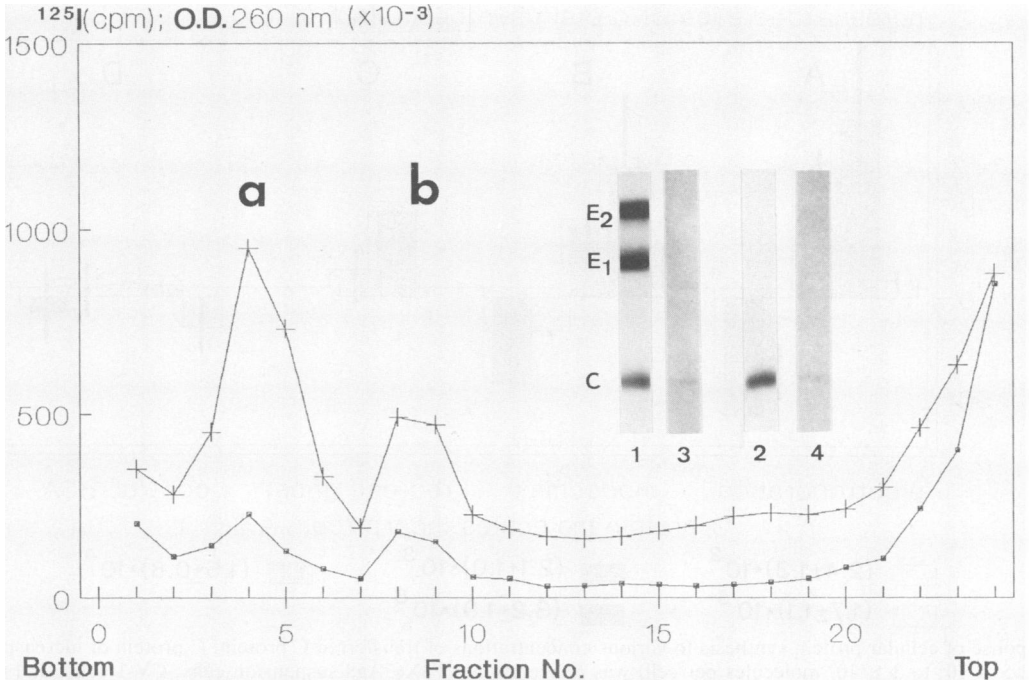

FIG. 3. Biological activity of liposome-delivered C protein. Vero cells (about 3×10^6 cells) were infected with SFV. They were then exposed at 5 h postinfection to liposomes in which ^{125}I-labeled C protein was encapsulated (for details, see the text). The progeny virus was subjected to polyethylene glycol 6000 precipitation, followed by ultracentrifugation through a 20 to 60% discontinuous sucrose gradient. The purified virus was then treated with the zwitterionic detergent CHAPS and overlayed onto a 10 to 30% sucrose gradient in TN buffer containing a layer of 60% sucrose. Centrifugation was for 2.5 h at 145,000 × *g* at 4°C in an SW41 rotor. Fractions (0.5 ml each) were collected from above and analyzed for optical densities (O.D.) at 260 nm (+) and for radioactivity (■) in a gamma counter. The inset shows the SDS-PAGE analyses (10 to 15% gradient gels under nonreducing conditions) of the virus (a) and the nucleocapsids (b). Lanes 3 and 4, Autoradiographs of the ^{125}I-labeled virus and nucleocapsids, respectively; lanes 1 and 2, autoradiographs of a similarly treated control virus preparation; however, the protein was labeled with [^{35}S]methionine. E_1 and E_2, Envelope proteins with M_rs of 49,000 and 52,000, respectively; C, C protein with an M_r of 33,000.

time course of repression of protein synthesis was investigated, we found that, similar to induction, repression lasted only for about 3 to 4 h following the delivery of C protein to the target cell (data not shown).

SDS-PAGE analysis of proteins following induction with liposome-delivered C protein. To reveal whether low concentrations of C protein are responsible for triggering an overall stimulation of protein synthesis or, alternatively, the synthesis of particular proteins was enhanced, total proteins of delivered and control cells were analyzed on SDS-10 to 15% polyacrylamide gradient gels. The autoradiographs of the protein patterns of delivered, induced cells (Fig. 6b) and control cells (Fig. 6a) are shown. Following densitometric scanning of the autoradiographs, the differences in the intensities of the various bands of the induced and control cells became even more apparent (Fig. 6c). The results suggest that proteins of the three distinct M_r size classes were induced, whereas other proteins remained unchanged. The first class which was induced was found to exhibit apparent M_rs of 70,000 and higher; the second class comprised proteins with intermediate M_rs ranging between 45,000 and 60,000, whereas proteins of the low M_r class were found to have M_rs of 27,000 and lower. Unchanged, major proteins were found to have M_rs of 75,000, 58,000, and 43,000, with the last two species probably representing tubulin and actin, respectively. Several unchanged minor proteins with M_rs ranging between 40,000 and 30,000 were also observed.

SDS-PAGE analysis of proteins following repression with delivered C protein. Figure 7 depicts the superimposed scans of two autoradiographs showing the protein patterns of C-protein-delivered, repressed cells and control cells. The result suggests that in contrast to low amounts of transferred C protein, high concentrations (about 10^6 molecules per cell) led to an overall repression of protein synthesis.

DISCUSSION

Earlier studies have suggested that in a cell-free in vitro translation system C-protein molecules are responsible for the shutoff of host protein synthesis (30). The results obtained in our laboratory showing that high concentrations of transferred C protein (10^5 to 10^6 molecules per cell) significantly repress protein synthesis support this finding. In addition, they are in line with the earlier report that SFV causes the shutoff of the host protein synthesis which takes place between 3 and 5 h after infection and leads to the exclusive production of viral structural proteins (7).

On the other hand, previous studies have shown that several lytic viruses, including paramyxoviruses (5, 22), herpesviruses (20), adenoviruses (19), papovaviruses (15), rhabdoviruses, and togaviruses (9), induce the synthesis of host proteins which are related to the so-called stress or heat shock proteins early during infection. All of these experiments were done with multiplicities of infection varying between 5 and 50 PFU per cell. We therefore were interested to examine the impact of low concentrations of C-protein molecules in an in vivo system which would mimic a

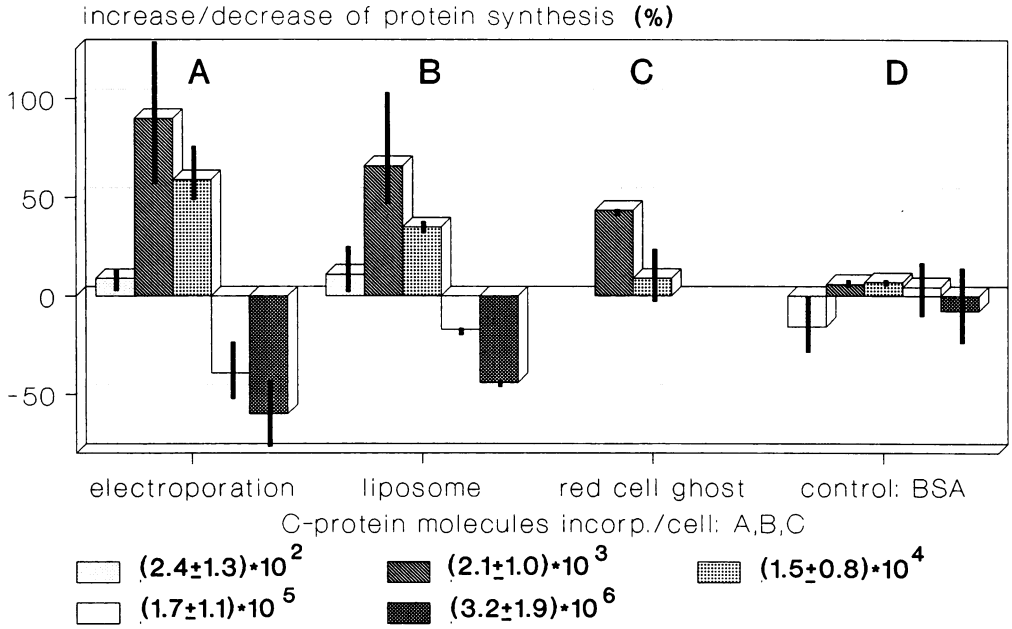

FIG. 4. Response of cellular protein synthesis to various concentrations of transferred C protein. C protein of increasing concentrations (ranging from 2.5×10^2 to 3×10^6 molecules per cell) was delivered into P3X63Ag8 suspension cells, CV-1 cells, and GpBind4 cells by electroporation- (A), liposome- (B), and erythrocyte-ghost-mediated delivery (C), as described in the text. (D) Electroporation-mediated delivery of increasing concentrations of bovine serum albumin (BSA). Following delivery, protein synthesis in the respective cells was assessed by incubating the cells in medium containing [^{35}S]methionine for 30 min at 37°C. Trichloroacetic acid-precipitable counts and protein content were determined as described in the text. The value for control cells was 0%. The values presented here are the arithmetic means of five experiments each for electroporation- and liposome-mediated delivery and two experiments for erythrocyte-ghost-mediated delivery.

low-input multiplicity of infection. To this end we studied protein synthesis as a response to low concentrations of C protein transferred into various target cells. The salient finding of our investigation was the observation that low concentrations of delivered C-protein molecules do not trigger a repression of protein synthesis but, in contrast, markedly induce protein synthesis. This was shown to be the case for three different cellular substrates, namely, P3X63Ag8 suspension cells, CV-1 cells, and GpBind4 cells. The induction of protein synthesis was shown to take place in a rather narrow concentration range (10^2 to 10^4 molecules per cell), with a maximum at about 10^3 molecules per cell. Taking into consideration that the nucleocapsid of SFV is made up of 180 C-protein molecules (8), then 10^3 molecules incorporated per cell corresponds to about five intact virions, or in other terms, to a low-input multiplicity of infection. In all three cell lines tested maximum induction was found to be at about 10^3 molecules per cell, whereas 10^4 molecules per cell gave a significantly lower response. The results also suggest that induction of protein synthesis does not result in an overall stimulation, but represents an augmentation of classes with specific M_rs. These classes comprise proteins with M_rs of 70,000 and higher, with M_rs ranging between 45,000 and 60,000, and finally M_rs of 27,000 and lower. Whether these proteins are indeed heat shock or stress proteins is being investigated in our laboratory and will be described in a future report.

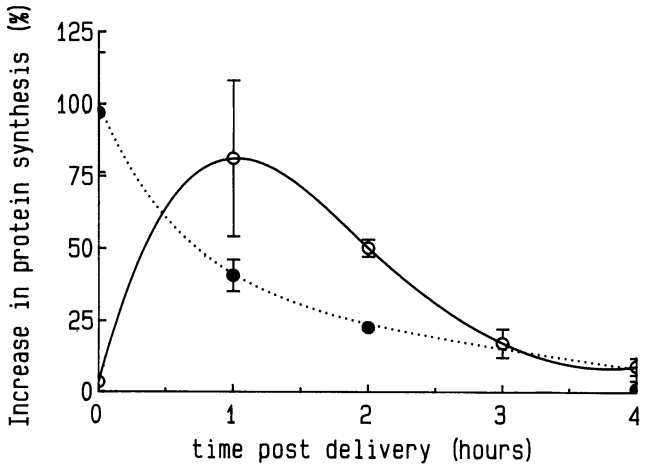

FIG. 5. Time course of induction of protein synthesis after challenge with low concentrations of C protein. C protein was delivered into P3X63Ag8 suspension cells (2×10^3 molecules per cell) and CV-1 cells (1.5×10^3 per cell) by electroporation (●) and liposomes (○), respectively. The cells were incubated at 37°C for various time periods, and incorporation of [^{35}S]methionine into newly synthesized protein after the various incubation periods was determined as described in the text. The value for control cells was 0%.

The critical concentration necessary to switch from induction to repression of protein synthesis was found to be somewhere between 10^4 and 10^5 molecules per cell. Whereas repression with 1×10^5 molecules per cell brought down protein synthesis by 39% (electroporation-mediated delivery), 3×10^6 molecules per cell resulted in an even higher inhibition (60%).

Maximum stimulation with electroporation-mediated delivery is apparently an immediate event, whereas with liposomes it is delayed by about 1 h. This delay can be explained

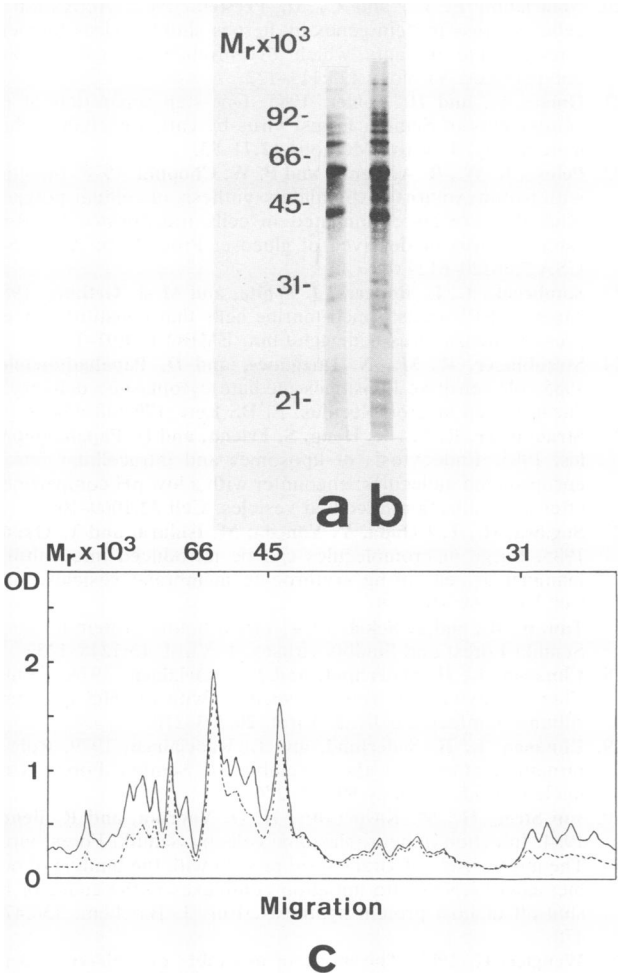

FIG. 6. SDS-PAGE analysis of proteins following induction with low amounts of liposome-delivered C protein. C protein was transferred into CV-1 cells (about 2×10^3 molecules per cell) by using liposome-mediated delivery. At 1 h after delivery, proteins were labeled for 30 min at 37°C with [^{35}S]methionine. Mock-treated (buffer-encapsulated liposomes) cells served as a control. After the labeling, the cells were lysed and samples containing equal concentrations of protein were analyzed on a 10 to 15% gradient gel, autoradiographed, and scanned as described in the text. Autoradiographs are of the protein patterns of induced (b) and control (a) cells. (c) Superimposed scans of the two autoradiographs. Symbols: —, Total proteins of induced cells; ----, total proteins of control cells. OD, Optical density.

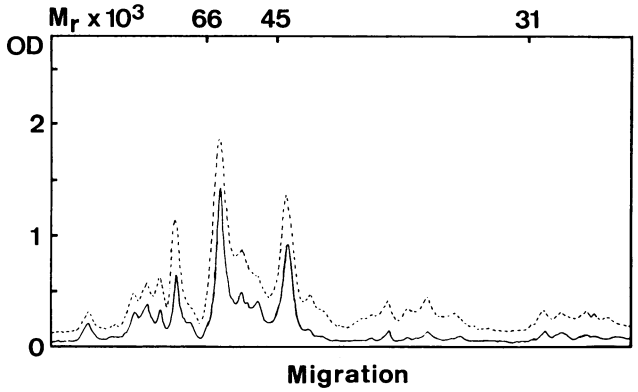

FIG. 7. SDS-PAGE analysis of proteins following repression with high amounts of transferred C protein. C protein was transferred into CV-1 cells (about 10^6 molecules per cell) by using liposome-mediated delivery. Labeling of the cells with [^{35}S]methionine and analysis of the protein patterns on SDS-PAGE were performed as described in the legend to Fig. 6. The figure shows the superimposed scans of two autoradiographs. Symbols: —, total proteins of repressed cells; ----, total proteins of control cells. OD, Optical density.

by the fact that delivery of C protein into the cytoplasmic compartment is mediated by the endocytotic pathway. It has been shown that it takes about 30 min for the vesicular stomatitis virus G protein to traverse the endocytotic pathway, i.e., from its uptake at the plasma membrane to the low pH-mediated fusion with the endosomal membrane (10).

The finding that stimulation and repression of protein synthesis depends on the amount of microinjected C protein per cell strongly suggests that our purification procedure left its biological activity intact. This was further supported by the evidence that ^{125}I-labeled C protein microinjected into SFV-infected cells actively participated in nucleocapsid formation and the maturation of progeny virus. We have ignored whether the observed stimulation and repression of cellular protein synthesis were due to the fact that the delivered C-protein molecules exerted their regulation on the level of transcription, translation, or both. One could reasonably argue that the kinetics of the stimulation and repression of protein synthesis favor the possibility of translational control; e.g., the cellular response toward the electroporation-mediated delivery of C-protein molecules is an immediate event and induction and repression occur for a limited time only. However, the epiphenomenon of a putative transcriptional control cannot be excluded.

In addition, the experiments outlined here show that electroporation-, liposome-, and erythrocyte-ghost-mediated deliveries of proteins represent useful tools for the elucidation of their action in target cells.

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