JOURNAL OF VIROLOGY, Mar. 1994, p. 1721–1727
0022-538X/94/$04.00+0

Vol. 68, No. 3

Comparison of the Effects of Sindbis Virus and Sindbis Virus Replicons on Host Cell Protein Synthesis and Cytopathogenicity in BHK Cells

ILYA FROLOV AND SONDRA SCHLESINGER*

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri

Received 15 October 1993/Accepted 13 December 1993

Infection of BHK cells by Sindbis virus leads to rapid inhibition of host cell protein synthesis and cytopathic effects (CPE). We have been studying these events to determine whether the expression of a specific viral gene is required and, in the present study, have focused our attention on the role of the structural proteins—the capsid protein and the two membrane glycoproteins. We tested a variety of Sindbis viruses and Sindbis virus replicons (virus particles containing an RNA that is self-replicating but with some or all of the viral structural protein genes deleted) for their abilities to inhibit host cell protein synthesis and cause CPE in infected BHK cells. Our results show that shutoff of host cell protein synthesis occurred in infected BHK cells when no viral structural proteins were synthesized and also under conditions in which the level of the viral subgenomic RNA was too low to be detected. These results support the conclusion that the early steps in viral gene expression are the ones required for the inhibition of host cell protein synthesis in BHK cells. In contrast, the Sindbis viruses and Sindbis virus replicons were clearly distinguished by the time at which CPE became evident. Viruses that synthesized high levels of the two membrane glycoproteins on the surface of the infected cells caused a rapid (12 to 16 h postinfection) appearance of CPE, and those that did not synthesize the glycoprotein spikes showed delayed (30 to 40 h) CPE.

Sindbis virus is the prototypic member of the *Alphavirus* genus of the *Togaviridae* family. Its replication strategy in cultured cells has been well characterized, in particular in the two cell systems in which the virus grows rapidly and to high titers—chicken embryo fibroblasts (CEF) and baby hamster kidney (BHK) cells (26, 28). Hallmarks of infection of these cells are the inhibition of host cell protein synthesis within a few hours after infection and cytopathic effects (CPE) occurring within 12 to 16 h postinfection (36). These responses to infection are typical for a wide range of different viruses, but their underlying causes are not well understood. Many viruses have devised strategies for inhibiting the synthesis of host proteins—strategies which at the same time permit translation of viral mRNAs. The prevailing ideas about the mechanisms of inhibition of host cell protein synthesis have been summarized in several reviews (12, 14, 27). They include competition by viral mRNAs for limiting translational factors, induction of factors that inhibit translation, inactivation of specific factors required for the translation of cellular mRNAs, degradation of cellular mRNAs, and alterations in the ionic environment of the cell affecting the translation of cellular mRNAs. Some of these may pertain only to a particular virus, and some viruses may employ more than one mechanism. These activities may also play a role in the death of infected cells. Recent studies with Sindbis virus have demonstrated that infection of several different cultured cell lines, including BHK cells, leads to apoptotic cell death (16).

We have been studying the infection of BHK cells by Sindbis virus in an effort to determine whether there are specific viral genes required for the shutoff of host cell protein synthesis and for CPE. The 12-kb genome of Sindbis virus is an RNA molecule of positive polarity which is capped at the 5′ terminus and polyadenylated at the 3′ terminus (26, 28). The 5′ two-thirds of the genome code for the nonstructural proteins required for transcription and replication of the viral RNA. The 3′ one-third of the genome contains the coding sequence for the viral structural proteins consisting of the capsid protein which interacts with genomic RNA to form the nucleocapsid, a hydrophobic 6-kDa (6K) protein, and the two membrane glycoproteins comprising the spikes inserted in the lipid envelope surrounding the nucleocapsid. In infected cells, the genomic RNA is translated to produce the nonstructural proteins; the structural proteins are translated from a subgenomic RNA (26S RNA) identical in sequence to the 3′ one-third of the genomic RNA and produced by transcription of the genomic length, minus strand from an internal promoter. In the past few years it has become possible to modify the genome of Sindbis virus through the use of recombinant DNA techniques (22). One modification has been to replace the structural protein genes with a heterologous gene so that the virus can be used as a vector for the expression of a heterologous protein (37). RNAs expressing the nonstructural proteins are self-replicating and can be packaged into virions when complemented by RNAs able to express the structural proteins (reviewed in references 2 and 25). Replicon genomes packaged into extracellular particles can infect cells efficiently but may express none or only some of the viral structural proteins. We have prepared a variety of different Sindbis virus replicons and have used them to investigate the ability of Sindbis virus to inhibit host cell protein synthesis and to cause CPE. We focused our attention on the role of the structural proteins and the subgenomic RNA in these events. Our data show that neither the synthesis of the structural proteins nor the synthesis of a subgenomic RNA affected the rate of shutoff of host protein synthesis in BHK cells. The expression of the

* Corresponding author. Mailing address: Department of Molecular Microbiology, Washington University School of Medicine, 660 S. Euclid Ave., Box 8230, St. Louis, MO 63110-1093. Phone: (314) 362-2746. Fax: (314) 362-1232.

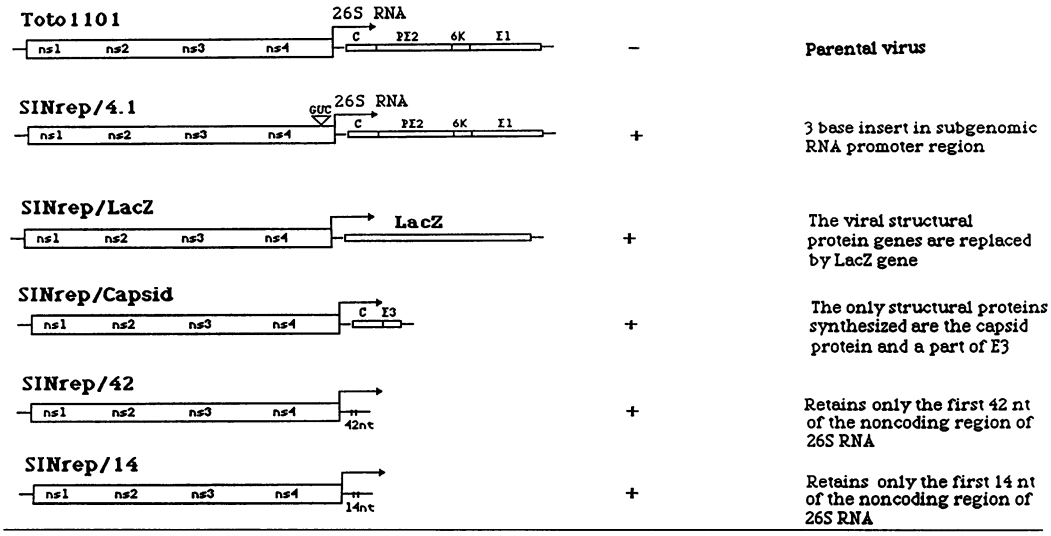

FIG. 1. Diagrams of Sindbis virus (Toto 1101) and Sindbis virus replicons. Toto 1101 (22) and SINrep/LacZ (3) have been described elsewhere. SINrep/4.1 is a newly cloned version of Toto1100CR4.1 (9) in which the nonstructural protein genes (upstream of the *Bam*HI site at position 7334 in the cDNA) were derived directly from the SINrep cDNA. This genome has a 3-base insertion (GUC) in the subgenomic RNA promoter between nt −4 and −5 of the promoter region (nt 1 is the start of the subgenomic RNA). SINrep/capsid contains the first 8,575 nt of the Sindbis virus genome and the 310 3′ nt. It codes for the complete capsid gene and the first 46 amino acids of PE2. SINrep/42 contains the first 7,646 nt of the Sindbis virus genome followed by a polylinker, the 3′-terminal 310 nt of the Sindbis virus genome plus 37 A residues. SINrep/14 differs from SINrep/42 in having only the first 14 nucleotides of the 26S RNA sequences.

viral glycoproteins, however, did affect the time of appearance of CPE in these cells.

MATERIALS AND METHODS

Sindbis viruses and Sindbis virus replicons. Sindbis viruses and replicons used in the study of protein synthesis are listed in Fig. 1. The nonstructural protein genes of the SINrep viruses shown here were all derived from SINrep/42 cDNA to ensure that they were identical. The other constructs are described in Fig. 6A.

Transcription, transfections, and packaging of the replicons. The transcription, transfection, and packaging methods have been described elsewhere (17, 18, 22). Transcriptions were carried out in the presence of the 5′ cap analog 7mG(5′)ppp(5′G) (New England Biolabs). The RNAs were introduced into cells by electroporation as described by Liljeström et al. (19). Two micrograms of each of the RNAs was used for electroporation. The SINrep RNAs and the RNA transcribed from pToto $E2C_{415}C_{416}$ were electroporated with the defective helper (DH) RNA, DH-BB(5′SIN) (3).

Assays for the titers of Sindbis virus replicons. The concentration of infectious particles expressing β-galactosidase had been determined by indirect immunofluorescence of infected cells using antibodies directed against β-galactosidase (3). We have developed two general assays which were tested with SINrep/LacZ. All three assays gave comparable titers for SINrep/LacZ.

(i) Assay on CEF. CEF infected with Sindbis virus replicons show clear evidence of CPE at an earlier time than BHK cells, and this is the basis of an assay for infectious units. Secondary cultures of CEF (either 5×10^5 or 10^6 cells per well) were seeded into six-well Costar dishes in minimal essential medium (MEM) containing 3% fetal calf serum. Four hours later, the cells were infected with serial dilutions of replicon samples. After a 1-h adsorption period, the liquid was replaced by MEM containing 1% fetal calf serum and 0.75% agarose. After 16 to 18 h, the cells were observed under the microscope and divided as follows (percent CPE): 0, 10 to 20, 40 to 50, 70 to 80, and 100. The concentration of replicon particles was calculated on the basis of the percent dead cells and the sample dilution. To increase the accuracy of this assay, we made 3-fold (instead of 10-fold) serial dilutions and determined the titer of each sample twice, once for each concentration of CEF. The titers of most samples were determined by this assay and by the luciferase assay. Both assays gave the same titers.

(ii) Luciferase assay. We have described a stably transformed cell line (BHKSINLuc2) that contains a defective Sindbis virus genome under the control of a Rous sarcoma virus promoter and the luciferase gene downstream of the viral subgenomic RNA promoter (21). Under defined conditions, the luciferase activity is proportional to the concentration of replicon added, and the titer of an unknown sample is determined on the basis of a standard curve.

RESULTS

Effects of Sindbis viruses and Sindbis virus replicons on the synthesis of host proteins. The viruses and replicons that were analyzed for their abilities to interfere with cellular protein synthesis in infected BHK cells are listed in Fig. 1. Toto 1101 and SINrep/4.1 contain the coding sequences for all of the

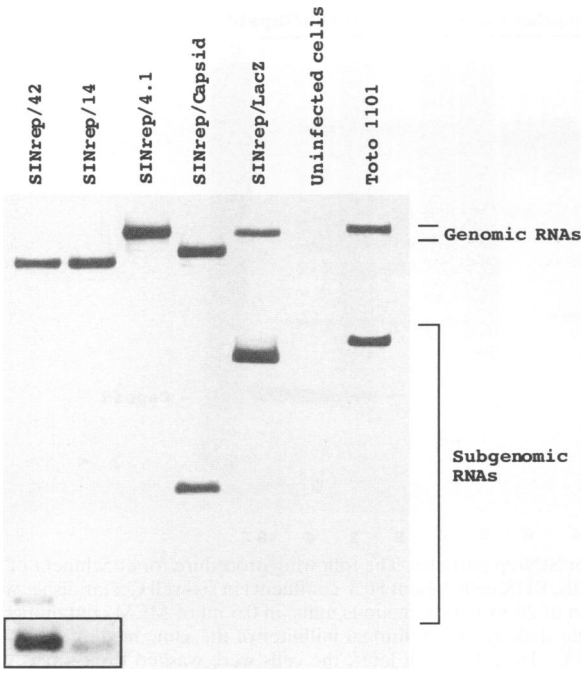

FIG. 2. RNAs synthesized in cells infected with Sindbis virus or SINrep particles. BHK cells (5×10^5) were infected with 50 PFU of Sindbis virus or 50 infectious units of a Sindbis virus replicon per cell and incubated with dactinomycin (1 μg/ml) and [^{3}H]uridine (20 μCi/ml) from 1 to 5 h postinfection. RNA was isolated from infected and control cells by using RNAzol B as described by the manufacturer (Tel-Test, Inc., Friendswood, Tex.). The radiolabeled RNAs were analyzed by agarose gel electrophoresis following denaturation with glyoxal in dimethyl sulfoxide. The virus or replicon sample used for infection is indicated at the top. The subgenomic RNAs produced in cells infected with SINrep/42 or SINrep/14 were detected by exposing the gel three times longer (box).

structural protein genes, but only cells infected with Toto 1101 produce the subgenomic 26S RNA and the structural proteins in normal amounts. SINrep/4.1 has a mutation in the promoter for transcription of the subgenomic RNA, and cells infected with this virus do not synthesize detectable levels of subgenomic RNA (9). Extracellular particles of this virus were obtained by packaging of the transcribed SINrep/4.1 RNA using a DH RNA (3).

The Sindbis virus replicons do not contain all of the coding sequences of 26S RNA. One (SINrep/capsid) lacked the viral glycoprotein genes; only the capsid protein was translated from the subgenomic RNA. Another had replaced the structural protein genes with the *lacZ* gene (3). The third and fourth contain the 5′ 42 or the 5′ 14 nucleotides (nt) of 26S RNA, respectively, followed by a polylinker and the 310 nt of the 3′ terminus of the virus genome. It has been proposed that the secondary structure of the 5′-terminal 50 nt of the alphavirus subgenomic RNA is an important feature in the ability of this RNA to bind translational initiation factors efficiently (1), and this structure should not exist in SINrep/14. All of these transcribed replicon RNAs were packaged by using a DH RNA that is not packaged (3).

BHK cells infected with equal amounts of the different viruses were analyzed for the synthesis of virus-specific RNA (Fig. 2). Comparable levels of Sindbis virus and SINrep RNAs were synthesized in the infected cells, and as expected, the level of subgenomic RNA could not be detected in cells infected with SINrep/4.1. DH RNAs were not detected, providing further evidence that the DH genomes were not packaged to any significant extent.

Before examining how infection of BHK cells by these different viruses and replicons affected the synthesis of host proteins, we established conditions under which the attachment of Sindbis virus to cells led to a synchronous infection. Under our standard conditions of attachment (multiplicity of infection of 20 and 1 h at 37°C), by 4 h postinfection only 20 to 40% of the BHK cells were scored as strongly positive in an indirect immunofluorescence assay in which the cells were fixed with methanol and then stained with antibodies directed against the viral structural proteins (data not shown). If the attachment step was carried out in the cold with continuous shaking of the dishes (see the legend to Fig. 3), essentially all of the cells scored as positive in this assay. The incorporation of [^{35}S]methionine into protein was analyzed by polyacrylamide gel electrophoresis at three different times after infection (Fig. 3). We compared the rates of shutoff of host protein synthesis by quantitating the amount of radioactivity in the sections of the gel containing only host proteins (Fig. 3). Within the limits of this method, no differences in the rates at which cellular protein synthesis was inhibited could be seen. Only three examples are shown in Fig. 4.

These data show that neither the synthesis of the viral structural proteins nor high levels of subgenomic RNA were essential for host cell protein synthesis to be inhibited. They did not, however, rule out the possibility that the incoming structural proteins might be involved in the shutoff of host cell protein synthesis. That alternative seemed unlikely on the basis of earlier reports that temperature-sensitive (*ts*) mutants which did not synthesize viral RNAs did not shut off host cell protein synthesis at nonpermissive temperatures (reviewed in reference 36). We obtained the same results with a *ts*11 mutant of Sindbis virus, which has a defect in the gene that codes for nsP1 and was derived from a cDNA clone in which the mutation had been placed in a Toto 1101 background (10). Virus entry and uncoating steps occur at the nonpermissive temperature, and the incoming proteins should be available to perform any putative function they had.

Effects of Sindbis viruses and Sindbis virus replicons on CPE in BHK cells. One notable difference between Sindbis virus Toto 1101 and the replicons listed in Fig. 1 was the time when CPE was detected. BHK cells infected with Toto 1101 showed extensive CPE by 12 to 16 h postinfection. In contrast, cells infected with any of the replicons did not show this type of CPE until 36 to 40 h after infection (Fig. 5). In addition to the samples listed in Fig. 1, we tested three others for their abilities to produce CPE in BHK cells. One was Sindbis virus TotoE2$C_{415}C_{416}$, in which cysteines 415 and 416 in the cytoplasmic tail of the glycoprotein E2 had been changed to serine and alanine, respectively (Fig. 6A). Cells infected with this virus provided an example in which the viral structural proteins were synthesized (Fig. 6B, lanes 6 and 7) and the glycoproteins were correctly inserted into the plasma membrane but the cells did not produce virus particles (data not shown). This virus was indistinguishable from wild-type Sindbis virus in its ability to produce CPE in BHK cells, indicating that it was not the production of virus particles that led to the early appearance of cytopathogenicity.

Cells infected with Sindbis virus synthesize two viral transmembrane glycoproteins—PE2, the precursor of E2, and E1. PE2 and E1 form a heterodimer which is transported to the plasma membrane (24). PE2 is cleaved to E2 en route to the cell surface and the E1-E2 heterodimers in the form of trimers constitute the spikes on the surface of virions. We asked

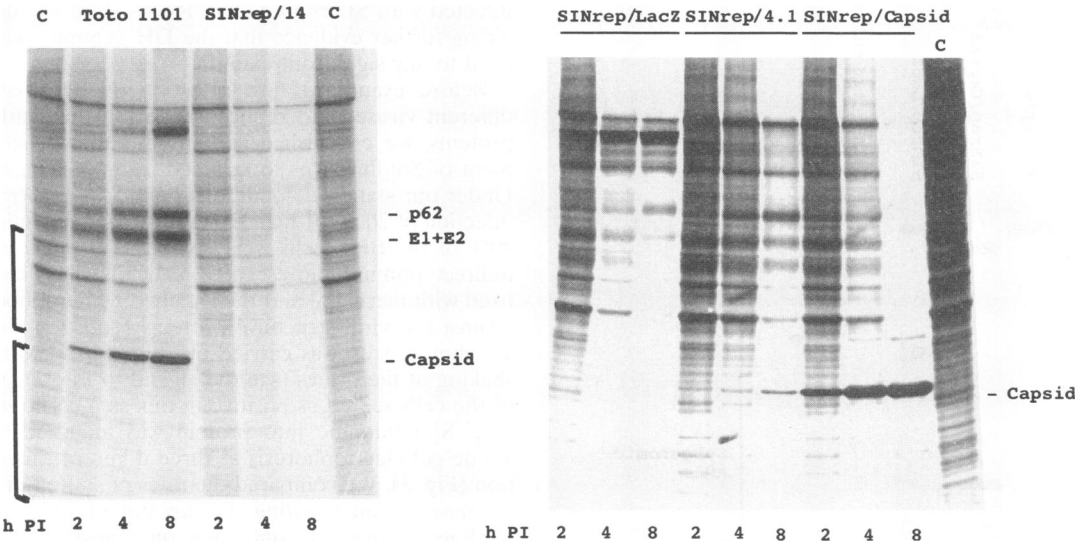

FIG. 3. [^{35}S]methionine-labeled proteins in cells infected with Sindbis virus or SINrep particles. The following procedure for attachment of the virus to cells was adopted to give reproducible synchronous infections of BHK cells. BHK cells (about 80% confluent) in six-well Costar dishes were infected with Sindbis virus or Sindbis virus replicons at multiplicities of infection of 20 PFU or infectious units, in 0.5 ml of MEM containing 2% fetal calf serum. The dishes were incubated at 8°C for 2 h with continuous gentle shaking. An additional milliliter of the same medium (at 37°C) was added to each well, and the dishes were placed in a CO_2 incubator at 37°C. Two, 4, or 8 h later, the cells were washed three times with phosphate-buffered saline (PBS) and incubated for 15 min at 37°C in medium lacking methionine. This medium was then replaced with 1 ml of the same medium containing 10 μCi of [^{35}S]methionine. After 20 min, 1 ml of MEM was added to each well, and the incubation continued for another 10 min. This dilution was sufficient to inhibit further incorporation of radioactive label into protein and significantly decreased the background of free [^{35}S]methionine in the polyacrylamide gels. The medium was then removed from the well, and the cells were washed three times with PBS and were scraped from the dish into PBS, pelleted by centrifugation, and dissolved in 25 μl of loading buffer (0.06 M Tris-HCl [pH 6.7], 2% sodium dodecyl sulfate, 5% β-mercaptoethanol, 5% glycerol, 0.05% bromophenol blue). One-fifth of the sample was analyzed on the gel. After electrophoresis, the gels were stained with Coomassie brilliant blue R, dried, and autoradiographed. The brackets indicate the regions that were analyzed to determine the level of host cell protein synthesis (Fig. 4). C, control; PI, postinfection.

whether the expression of either glycoprotein by itself would still lead to the more rapid CPE seen when both glycoproteins were expressed together. cDNAs with deletions in either the E2 or the E1 gene are illustrated in Fig. 6A. BHK cells infected with SINrep/E1 synthesized E1 and a small polypeptide (PE2*) that migrated on polyacrylamide gels slightly more slowly than the capsid protein (Fig. 6B, lanes 2 and 3). This small polypeptide is the size predicted for PE2* on the basis of the deletion made in the cDNA of Toto 1101. Cells infected with SINrep/E2 synthesized PE2 which was cleaved to E2; no E1 was detected (Fig. 6B, lanes 4 and 5). Identification of each of the glycoproteins was confirmed by immunoprecipitation experiments with both monospecific and polyclonal antibodies (data not shown).

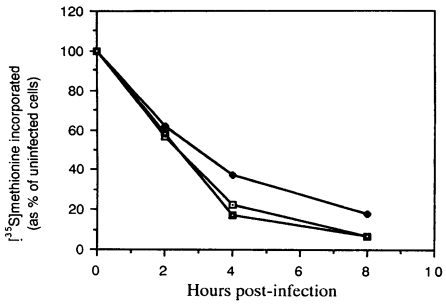

FIG. 4. Synthesis of host cell proteins at 2, 4, and 8 h after infection of BHK cells with Sindbis virus or Sindbis virus replicons. These data were obtained by analysis of the sections of the gels indicated in Fig. 3. Cells were infected with Sindbis virus (◆), SINrep/14 (⊡), or SINrep/LacZ (■). The rate of inhibition of host cell protein synthesis in the other samples was essentially the same as that shown.

Cells infected either with SINrep/E1 or with SINrep/E2 showed delayed CPE, indicating that the synthesis of either glycoprotein alone was not sufficient to produce an early onset of CPE. We carried out several pulse-chase experiments to determine whether one of the viral glycoproteins expressed in the absence of its partner would be degraded, but in both cases the proteins were stable. Figure 6B shows the results of one of the pulse-chase experiments. One reason that expression of PE2/E2 or E1 alone did not lead to rapid CPE could be that they were unable to reach the cell surface. Using virus specific antibodies, we determined the fraction of [^{35}S]methionine pulse-labeled viral glycoproteins that reached the outer cell surface after a 90-min chase period (8). The fraction of E2 on the surface of cells infected with SINrep/E2 was the same as that in cells infected with Sindbis virus. E1 was not found on the surface of cells infected with SINrep/E1 under conditions in which 5% of the E1 was on the surface in Sindbis virus-infected cells (data not shown). Therefore, the delayed appearance of CPE may be correlated with a lack of expression either of the E1-E2 heterodimer or of E1 itself on the cell surface.

DISCUSSION

Inhibition of host cell protein synthesis. Several mechanisms have been proposed for the inhibition of host cell protein synthesis by alphaviruses (12, 36). Some, such as a reduction in intracellular ribonucleoside triphosphate pools due to viral RNA synthesis (36), alterations in the ionic

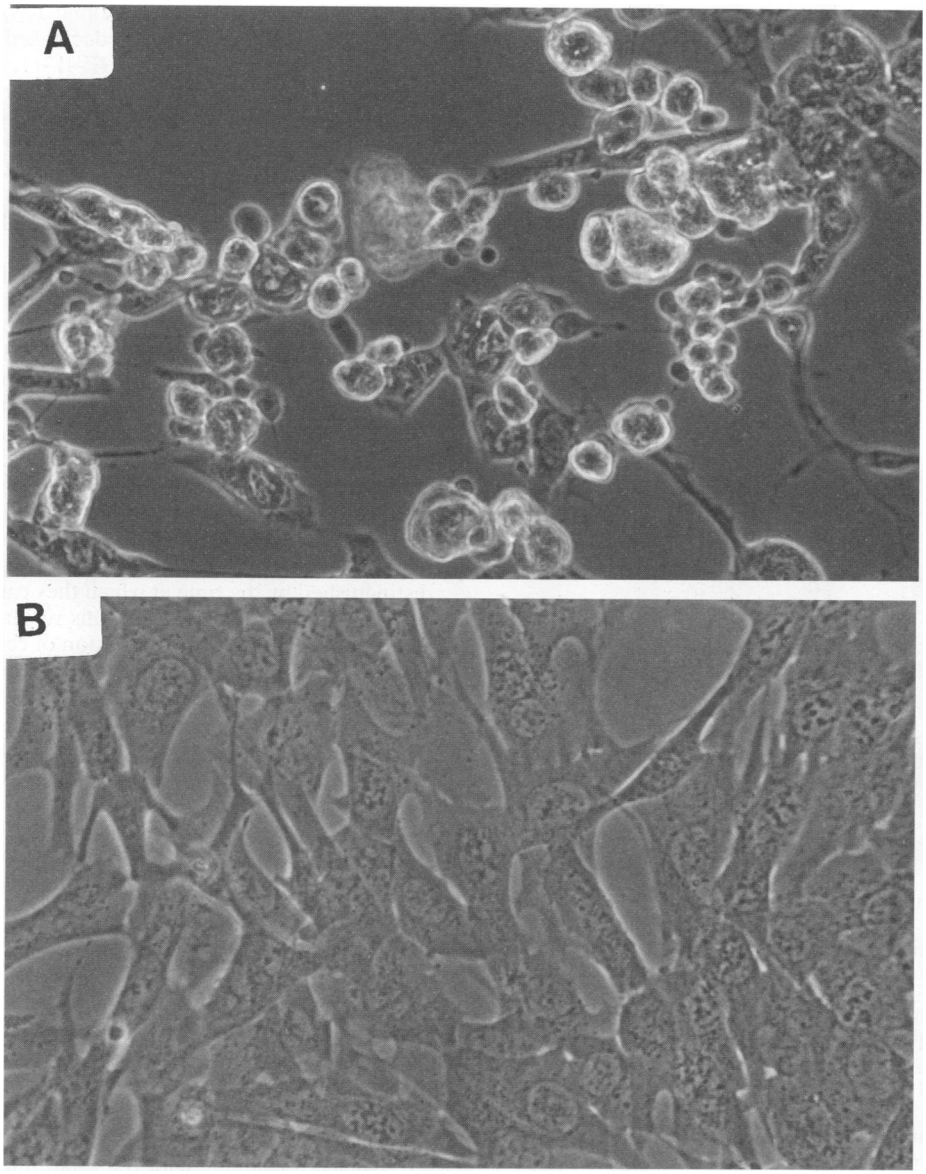

FIG. 5. BHK cells 16 h after infection with Sindbis virus Toto 1101 (A) or SINrep/capsid (B).

environment of the cell affecting the synthesis of host mRNAs more drastically than viral mRNAs (5, 12), and competition between viral and host mRNAs for ribosomes and host cell factors (12, 36), are mechanisms that may exert their effects during the infection of cells by a number of different RNA viruses (12, 14, 27). It has also been suggested that the alphavirus capsid protein is involved in the shutoff of host protein synthesis. The Semliki Forest virus capsid protein interferes with the binding of host mRNAs into 80S ribosomal initiation complexes (32). Introduction of this capsid protein directly into cells by electroporation led, at high concentrations, to an inhibition of host cell protein synthesis (6). Our studies with SINrep viruses, however, show that in BHK cells inhibition of host cell protein synthesis occurred under conditions in which neither the viral structural proteins nor the subgenomic RNA was synthesized. They provide strong evidence that it is the early events in viral gene expression that are essential for inhibiting host cell protein synthesis in BHK cells. Several other studies had not found a direct correlation between levels of the capsid protein and inhibition of cellular protein synthesis (23, 29). The authors of one of these studies (23) suggest that a mutation in the 6K protein gene prevented the ribosomes from translating the altered region of the 26S mRNA efficiently and that this resulted in more ribosomes being available for translation of cellular mRNAs. An alternative explanation is that small differences in the total number of cells infected (or in the synchrony of infection) could explain these results. Biochemical assays that measure early events in viral infection would probably not detect a small difference in the number of uninfected cells, but if some small fraction of the cells (for example 10%) infected by the mutationally altered virus remained uninfected, they might be detected in an assay that measured the synthesis of host cell proteins.

There have been extensive studies on the role of the double-stranded RNA-activated inhibitor (the p68 protein kinase) in inhibition of host cell protein synthesis after virus infection (reviewed in references 11, 13, and 20). This kinase is

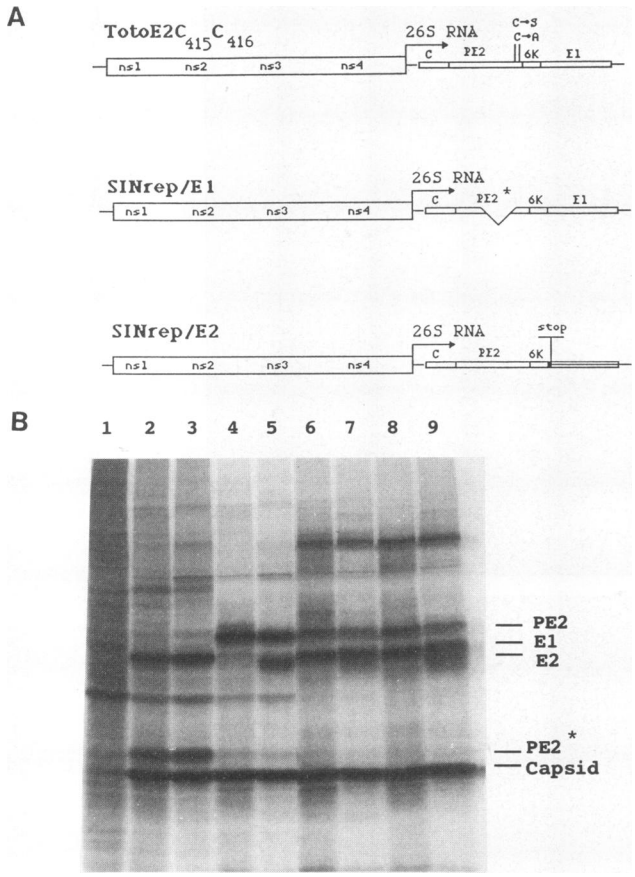

FIG. 6. (A) Diagrams of Sindbis virus, TotoE2C$_{415}$C$_{416}$, SINrep/E1, and SINrep/E2. pTotoE2C$_{415}$C$_{416}$ was constructed by K. G. Nitschko and M. Schlesinger. Site-specific mutations in the E2 protein gene changed the cysteine at position 415 to serine and the cysteine at position 416 to alanine. SINrep/E1 was derived from Toto 1101 by deletion of nt 8814 to 9615 of the genome, with the downstream coding region kept in frame. This deletion corresponded to amino acids 159 to 292 of PE2. SINrep/E2 was derived from Toto 1101 by deletion of nucleotides 10,038 to 10,273, which removed the nine carboxy terminal amino acids of the 6K protein and connected the remaining amino-terminal portion to an additional (but out-of-frame) 35 amino acids followed by a stop codon. (B) Pulse-chase labeling of BHK cells infected with Sindbis virus, TotoE2C$_{415}$C$_{416}$, SINrep/E1, or SINrep/E2. The procedures for infection and pulse-labeling are described in the legend to Fig. 3. Cells were labeled 8 h postinfection. After the 20-min pulse, the cells were either harvested (lanes 1, 2, 4, 6, and 8) or washed twice with warm MEM and then chased with complete MEM for 90 min before being harvested (lanes 3, 5, 7, and 9). Lane 1, uninfected cells; lanes 2 and 3, cells infected with SINrep/E1; lanes 4 and 5, cells infected with SINrep/E2; lanes 6 and 7, cells infected with TotoE2C$_{415}$C$_{416}$; lanes 8 and 9, cells infected with Toto 1101.

induced by interferon and is activated by double-stranded RNAs. After activation, it is autophosphorylated and phosphorylates the eucaryotic initiation factor eIF2α. This inactivates the initiation factor and leads to an overall inhibition of protein synthesis. Cellular protein synthesis is usually affected to a greater extent than virus-specified protein synthesis. Some viruses have devised strategies for overcoming this inhibition (13, 20, 27). Furthermore, viral mRNAs may be able to bind eucaryotic initiation factors with a greater affinity than most host mRNAs, and limiting amounts of an initiation factor would be sequestered by the viral mRNA-ribosome complexes. In this regard, the 26S subgenomic RNA of Semliki Forest virus has been shown to have a decreased requirement for the initiation factors eIF-4B and eIF-4F (1, 33, 34). This higher affinity may extend to other initiation factors, such as eIF2α. The effect of infection by Sindbis virus on the levels of the double-stranded RNA-activated kinase in BHK cells has not been studied. How infections by Sindbis virus and by the viral replicons affect the activity of this enzyme may shed some light on its potential role in inhibiting host cell protein synthesis in these cells.

We are continually learning of the multiple mechanisms that may come into play in the efforts of a virus to interfere with cellular metabolism and the inhibition of host cell protein synthesis. Our results establish that, in BHK cells, the synthesis of virus-specific genomic RNA and/or the synthesis of the nonstructural viral proteins is sufficient to inhibit cellular protein synthesis. We emphasize, however, that our data do not rule out the possibility that in some cells and under some conditions the synthesis of the subgenomic RNA or the structural proteins may also affect host cell protein synthesis.

Development of CPE in cells infected by Sindbis virus. Sindbis virus and the Sindbis virus replicons were clearly distinguished by the time at which they caused CPE in infected BHK cells. We conclude from this result that CPE was not a direct consequence of the inhibition of cellular protein synthesis—a conclusion also noted by Ulug and Bose (29). Viruses that synthesized high levels of the envelope glycoproteins on the surfaces of the infected cells caused a rapid (12 to 16 h postinfection) appearance of CPE; replicons that did not synthesize the glycoprotein spikes showed delayed (30 to 40 h) CPE. Ulug and Bose had reported that CPE was significantly delayed under conditions in which the viral glycoproteins were not correctly inserted into the cellular membranes (29). They showed that treatment of cells with tunicamycin which inhibited both glycosylation of the viral glycoproteins and the terminal steps in virus assembly prevented the normal development of CPE. Earlier studies had also shown that BHK cells treated with tunicamycin were unable to glycosylate the Sindbis virus glycoproteins and that these proteins were probably not correctly inserted into the infected cell plasma membrane (15). Although the formation of Sindbis virus particles is not required for the rapid onset of CPE, our results, taken together with those of Ulug and Bose (29) and Ulug et al. (30), indicate that the expression of both viral glycoproteins on the cell surface was correlated with the rapid onset of CPE. Ulug and Bose (29) and Ulug et al. (30, 31) had shown that infection of avian cells by Sindbis virus led to inhibition of the Na^+K^+ ATPase and proposed that a decrease in this activity and the disruption of the membrane integrity by the virus may contribute to the CPE. The mechanism by which virus infection inhibits the ATPase activity is not known, and other membrane-localized activities may also be disrupted after virus infection.

Growth of alphaviruses does not necessarily lead to cell death. It is well known that infection of insect cells by alphaviruses may not cause cytopathogenicity (4). Furthermore, a variant of Sindbis virus (SIN-1) isolated from BHK cells persistently infected with Sindbis virus for 1 month readily establishes persistent infections in BHK cells (35). The original persistent infection could be established only by infecting BHK cells with wild-type Sindbis virus in the presence of high levels of defective interfering particles. SIN-1 establishes a persistent infection in the absence of defective interfering particles, although their role in maintaining the persistent state is not known. The lack of CPE in BHK cells infected with SIN-1 may be due to a lower rate of synthesis of virus-specific products,

but it is also possible that one or more of the virus genes have changed in some way, mitigating both the inhibition of host cell protein synthesis and cell death (7).

Recent studies have shown that a rat prostatic adenocarcinoma cell line expressing high levels of the cellular oncogene *bcl-2* is protected against the CPE of infection by Sindbis virus (16). Similar results were not obtained with BHK cell lines carrying the *bcl-2* gene (7), and questions of the role of the cell and that of Sindbis virus in cell death as well as in the inhibition of protein synthesis remain to be answered.

ACKNOWLEDGMENTS

This research was supported by grant AI11377 from the National Institute of Allergy and Infectious Diseases.

We thank Milton Schlesinger for providing the cDNA for TotoE2$C_{415}C_{416}$, Mary Jones for the *ts*11 clone, and David Sibley for helping with the photographs.

REFERENCES

1. **Berben-Bloemheuvel, G., M. A. M. Kasperaitis, H. van Heugten, A. A. M. Thomas, H. van Steeg, and H. O. Voorma.** 1992. Interaction of initiation factors with the cap structure of chimaeric mRNA containing the 5′-untranslated regions of Semliki Forest virus RNA is related to translational efficiency. Eur. J. Biochem. **208:**581–587.
2. **Bredenbeek, P., and C. M. Rice.** 1992. Animal RNA virus expression systems. Semin. Virol. **3:**297–310.
3. **Bredenbeek, P. J., I. Frolov, C. M. Rice, and S. Schlesinger.** 1993. Sindbis virus expression vectors: packaging of RNA replicons by using defective helper RNAs. J. Virol. **67:**6439–6446.
4. **Brown, D. T., and L. D. Condreay.** 1986. Replication of alphaviruses in mosquito cells, p. 171–207. *In* S. Schlesinger and M. J. Schlesinger (ed.), The Togaviridae and Flaviviridae. Plenum Press, New York.
5. **Contreras, A., and L. Carrasco.** 1979. Selective inhibition of protein synthesis in virus-infected mammalian cells. J. Virol. **29:**114–122.
6. **Elgizoli, M., Y. Dai, C. Kempf, H. Koblet, and M. R. Michel.** 1989. Semliki Forest virus capsid protein acts as a pleiotropic regulator of host cellular protein synthesis. J. Virol. **63:**2921–2928.
7. **Frolov, I., and S. Schlesinger.** Unpublished data.
8. **Gaedigk-Nitschko, K., and M. J. Schlesinger.** 1991. Site-directed mutations in Sindbis virus E2 glycoprotein's cytoplasmic domain and the 6K protein lead to similar defects in virus assembly and budding. Virology **183:**206–214.
9. **Grakoui, A., R. Levis, R. Raju, H. V. Huang, and C. M. Rice.** 1989. A *cis*-acting mutation in the Sindbis virus junction region which affects subgenomic RNA synthesis. J. Virol. **63:**5216–5227.
10. **Hahn, Y. S., E. G. Strauss, and J. H. Strauss.** 1989. Mapping of RNA^- temperature-sensitive mutants of Sindbis virus: assignment of complementation groups A, B, and G to nonstructural proteins. J. Virol. **63:**3142–3150.
11. **Hovanessian, A. G.** 1993. Interferon-induced dsRNA-activated protein kinase (PKR): antiproliferative, antiviral and antitumoral functions. Semin. Virol. **4:**237–245.
12. **Kääriäinen, L., and M. Ranki.** 1984. Inhibition of cell functions by RNA-virus infections. Annu. Rev. Microbiol. **38:**91–109.
13. **Katze, M. G.** 1993. Games viruses play: a strategic initiative against the interferon-induced dsRNA activated 68,000 Mr protein kinase. Semin. Virol. **4:**259–268.
14. **Kozak, M.** 1992. Regulation of translation in eukaryotic systems. Annu. Rev. Cell Biol. **8:**197–225.
15. **Leavitt, R., S. Schlesinger, and S. Kornfeld.** 1977. Impaired intracellular migration and altered solubility of nonglycosylated glycoproteins of vesicular stomatitis virus and Sindbis virus. J. Biol. Chem. **252:**9018–9023.
16. **Levine, B., Q. Huang, J. T. Isaacs, J. C. Reed, D. E. Griffin, and J. M. Hardwick.** 1993. Conversion of lytic to persistent alphavirus infection by the *bcl-2* cellular oncogene. Nature (London) **361:**739–742.
17. **Levis, R., B. G. Weiss, M. Tsiang, H. Huang, and S. Schlesinger.** 1986. Deletion mapping of Sindbis virus DI RNAs derived from cDNAs defines the sequences essential for replication and packaging. Cell **44:**137–145.
18. **Liljeström, P., and H. Garoff.** 1991. A new generation of animal cell expression vectors based on the Semliki Forest virus replicon. Bio/Technology **9:**1356–1361.
19. **Liljeström, P., S. Lusa, D. Huylebroeck, and H. Garoff.** 1991. In vitro mutagenesis of a full-length cDNA clone of Semliki Forest virus: the small 6,000-molecular-weight membrane protein modulates virus release. J. Virol. **65:**4107–4113.
20. **Mathews, M. B.** 1993. Viral evasion of cellular defense mechanisms: regulation of the protein kinase DAI by RNA effectors. Semin. Virol. **4:**247–257.
21. **Olivo, P., I. Frolov, and S. Schlesinger.** 1994. A cell line that expresses a reporter gene in response to infection by Sindbis virus: a prototype for detection of positive strand RNA viruses. Virology **198:**381–384.
22. **Rice, C. M., R. Levis, J. H. Strauss, and H. V. Huang.** 1987. Production of infectious RNA transcripts from Sindbis virus cDNA clones: mapping of lethal mutations, rescue of a temperature-sensitive marker, and in vitro mutagenesis to generate defined mutants. J. Virol. **61:**3809–3819.
23. **Schlesinger, M. J., S. D. London, and C. Ryan.** 1993. An in-frame insertion into the Sindbis virus 6K gene leads to defective proteolytic processing of the virus glycoproteins, a *trans*-dominant negative inhibition of normal virus formation, and interference in virus shut off of host-cell protein synthesis. Virology **193:**424–432.
24. **Schlesinger, M. J., and S. Schlesinger.** 1987. Domains of virus glycoproteins. Adv. Virus Res. **33:**1–44.
25. **Schlesinger, S.** 1993. Alphaviruses—vectors for the expression of heterologous genes. Trends Biotechnol. **11:**18–22.
26. **Schlesinger, S., and M. Schlesinger.** 1990. Replication of Togaviridae and Flaviviridae, p. 697–711. *In* B. N. Fields and D. M. Knipe (ed.), Virology. Raven Press, New York.
27. **Schneider, R. J., and T. Shenk.** 1987. Impact of virus infection on host cell protein synthesis. Annu. Rev. Biochem. **56:**317–332.
28. **Strauss, E. G., and J. H. Strauss.** 1986. Structure and replication of the alphavirus genome, p. 35–82. *In* S. Schlesinger and M. J. Schlesinger (ed.), The Togaviridae and Flaviviridae. Plenum Press, New York.
29. **Ulug, E. T., and H. R. Bose, Jr.** 1985. Effect of tunicamycin on the development of the cytopathic effect in Sindbis virus-infected avian fibroblasts. Virology **143:**546–557.
30. **Ulug, E. T., R. F. Garry, and H. R. Bose, Jr.** 1989. The role of monovalent cation transport in Sindbis virus maturation and release. Virology **172:**42–50.
31. **Ulug, E. T., R. F. Garry, M. R. F. Waite, and H. R. Bose, Jr.** 1984. Alterations in monovalent cation transport in Sindbis virus-infected chick cells. Virology **132:**118–130.
32. **van Steeg, H., M. Kasperaitis, H. O. Voorma, and R. Benne.** 1984. Infection of neuroblastoma cells by Semliki Forest virus. The interference of viral capsid protein with the binding of host messenger RNAs into initiation complexes is the cause of the shut-off of host protein synthesis. Eur. J. Biochem. **138:**473–478.
33. **van Steeg, H., A. Thomas, S. Verbeek, M. Kasperaitis, H. O. Voorma, and R. Benne.** 1981. Shutoff of neuroblastoma cell protein synthesis by Semliki Forest virus: loss of ability of crude initiation factors to recognize early Semliki Forest virus and host mRNA. J. Virol. **38:**728–736.
34. **van Steeg, H., M. van Grinsven, F. van Mamsfeld, H. O. Voorma, and R. Benne.** 1981. Initiation of protein synthesis in neuroblastoma cells infected by Semliki Forest virus. FEBS Lett. **129:**62–66.
35. **Weiss, B., R. Rosenthal, and S. Schlesinger.** 1980. Establishment and maintenance of persistent infection by Sindbis virus in BHK cells. J. Virol. **33:**463–474.
36. **Wengler, G.** 1980. Effects of alphaviruses on host cell macromolecular synthesis, p. 459–472. *In* R. W. Schlesinger (ed.), The togaviruses: biology, structure, replication. Academic Press, New York.
37. **Xiong, C., R. Levis, P. Shen, S. Schlesinger, C. M. Rice, and H. V. Huang.** 1989. Sindbis virus: an efficient, broad host range vector for gene expression in animal cells. Science **243:**1188–1191.