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Differential Inhibition of Host Protein Synthesis in L Cells Infected with RNA⁻ Temperature-Sensitive Mutants of Vesicular Stomatitis Virus

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The response of mouse L cells to infection with wild-type (*wt*) and temperature-sensitive (*ts*) mutants of vesicular stomatitis virus was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to delineate the synthesis of host cell and viral proteins. Experiments utilized transcriptase mutants of complementation group I (*ts*114 and *ts*13), a group IV mutant (*ts*44) that is restricted in total RNA synthesis (RNA⁻) but not in primary transcription, and a group II mutant (*ts*52) variably restricted in RNA synthesis (RNA±). L cells infected with *ts* mutants at permissive temperature exhibited the *wt* response of progressive inhibition of host cell protein synthesis accompanied by accumulation of all five viral proteins. Mutant *ts*44 (IV) also switched off cell protein synthesis at restrictive temperature and accumulated all five viral proteins, but with disproportionate ratios of N and G proteins. At restrictive temperature, cells infected with group I *ts* mutants failed to accumulate any viral protein and did not exhibit significant reduction in host cell protein synthesis. These data suggest that vesicular stomatitis virus inhibits cell protein synthesis at a stage of viral infection after transcription and possibly translation but preceding replication of progeny viral RNA.

Inhibition of host cell macromolecular synthesis by vesicular stomatitis (VS) virus has been demonstrated by a variety of experimental procedures (7, 10, 12, 26, 28). Two principal, but not mutually exclusive, hypotheses have been proposed by which VS virus alters host synthetic functions resulting in cell death. In one mechanism, inhibition of host macromolecular synthesis has been considered to be a direct consequence of a structural component of the invading virion (7, 10, 28), which need not be infectious, acting to perturb some surface membrane function (7). The results presented here and observations reported by others (12) suggest that this original hypothesis is now untenable. The second hypothesis suggests that synthesis of virus-specific products is essential for interrupting host macromolecular synthesis (12, 13, 27). Suggested as potential cytotoxic products, newly synthesized by the infecting virus, are viral proteins (13, 27) and intermediates of viral RNA replication (13).

We approached the question of the inhibitory effect of VS virus on host cell protein synthesis by examining the response of mouse L cells to infection with wild-type (*wt*) and RNA⁻ temperature-sensitive (*ts*) mutants of VS virus. Selected for this study were mutants from complementation group I, which are defective in primary and secondary transcription functions (4, 11, 15, 16, 21), and a group IV mutant, ostensibly defective in replication function but in which primary transcription is unimpaired (2, 17, 21, 22). Polyacrylamide gel electrophoretic profiles of proteins accumulating in virus-infected and uninfected cells were compared using computer-assisted difference analysis (8, 29). This analytical method permitted us to determine the extent to which host cell protein synthesis is inhibited in relation to simultaneous accumulation of virus-specific proteins.

MATERIALS AND METHODS

Viruses, cells, and media. The *wt* strain of the Indiana serotype of VS virus was originally obtained from the U. S. Agricultural Research Center, Beltsville, Md. (23). The *ts* mutants of VS virus designated *ts*G13 (I), *ts*G44 (IV), and *ts*G114 (I) were provided through the courtesy of C. R. Pringle, Institute of Virology, Glasgow, Scotland (17). The VS virus mutant designated *ts*052 (II) was kindly provided by A. Flamand and F. Lafay, Faculté des Sciences, Orsay, France (5). Virus stocks were prepared by growth in BHK-21 cells and titrated by assay of PFU on monolayers of L cells. Host cells for all experiments reported herein were L cells. Media and serum were obtained from Grand Island Biological Co., Grand Island, New York.

Radioisotopes and chemicals. [^{3}H]leucine (50 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. ^{14}C-labeled protein hydrolysate (55 mCi/matom) and Nuclear-Chicago solubilizer were obtained from Amersham/Searle, Arlington Heights, Ill. [^{3}H]uridine (20 Ci/mmol) was obtained from Schwarz/Mann, Orangeburg, N.Y. Nonidet P-40 (NP-40) was obtained from Shell Chemical Co., Ltd., London.

Virus stock preparation. Stocks of *wt* VS virus and *ts* mutants were prepared from recently cloned virus. Confluent monolayers of BHK-21 cells in Falcon plastic flasks (75-cm^2 surface) were inoculated at an input multiplicity of 0.1 PFU/cell, incubated at 31 C, and harvested at 18 h (*wt*) or 24 h (*ts* mutants) postinfection. These virus stock preparations, clarified by centrifugation to remove cell debris, were titrated by plaque assay on L-cell monolayers at 31 and 39 C and stored at −70 C. No revertants were detected by plaque assay at 39 C with any of the mutant stocks. Radioactively labeled virus, used as marker for ^{3}H-labeled protein analysis on polyacrylamide gels, was prepared in the same manner using BHK-21 medium, containing 1 μCi of ^{14}C-labeled protein hydrolysate per ml. Labeled virus was purified as previously described (3).

Preparation and analysis of radioactively labeled proteins in virus-infected and uninfected cells. Monolayers of ~10^6 L cells in Falcon plastic petri dishes (35 by 10 mm) were infected at an input multiplicity of ~100 PFU/cell. After a 60-min adsorption period at 31 C, the virus inoculum (0.2 ml) was removed. The monolayers were placed at 31 C or 39 C and overlaid with 1.5 ml of Eagle basal medium prewarmed to the desired temperature. This temperature shift was considered to be zero time. At prescribed intervals thereafter, 0.5 ml of prewarmed Eagle basal medium containing [^{3}H]leucine was added to virus-infected and uninfected monolayers at 31 and 39 C to give a final [^{3}H]leucine concentration of 10 μCi/ml. After a 60-min labeling period, the petri dishes were removed from the incubator and placed on an ice tray. The warm Eagle basal medium was replaced by ice-cold Earle balanced salt solution, and the cell sheet was removed with the aid of a rubber policeman. The cells were pelleted by centrifugation at 800 × *g* for 10 min at 4 C and washed twice with cold Earle balanced salt solution. The cell pellet was suspended in 1% NP-40 in Earle balanced salt solution (vol/vol) and repeatedly agitated on a Vortex mixer. The nuclei were removed by centrifugation at 800 × *g* for 10 min. An equal volume of ice-cold 20% trichloroacetic acid (wt/vol) was added, and precipitable materials were collected by centrifugation at 2,000 × *g* for 20 min at 4 C. The precipitate was washed once with 10% trichloroacetic acid, twice with acetone, and finally suspended in 100 μl of 0.1 M sodium phosphate buffer (pH 7.4) containing 2% sodium dodecyl sulfate (SDS) (wt/vol).

Constituent proteins of the entire 100-μl resuspension volume were analyzed by polyacrylamide gel electrophoresis. A small volume (5 μl) of ^{14}C-labeled VS virus marker was added to the constituent proteins in 2% SDS. The sample was made 1% with respect to 2-mercaptoethanol (vol/vol), and the proteins were dissociated by heating at 100 C for 2 min. The samples were layered over 7.5% SDS-polyacrylamide gels prepared as previously described (25) and subjected to electrophoresis at 4 mA/gel for 16 h.

Difference analysis of proteins synthesized in uninfected and VS virus-infected L cells. The difference analysis procedure, similar to that described by Hightower and Bratt (8), permitted the monitoring of the percentage of reduction in host cell protein accumulation and the accumulation of virus-specific proteins. The percentage of reduction in host cell protein accumulation was determined in the following manner. The region of each gel extending from fractions 15 to 30 was chosen to normalize activity because in this region no viral proteins were evident. The normalization ratio, indicating the degree of reduction of host cell protein accumulation, was determined by using the expression: $I/U = N$, where I is the sum of ^{3}H activity (counts per minute) in gel fractions 15 to 30 from virus-infected cells and U is the sum of ^{3}H activity (counts per minute) in gel fractions 15 to 30 from uninfected cells. Thus, 100 N is the reduction in host-specific protein accumulation in virus-infected cells expressed as a percentage of the uninfected cell protein accumulation.

The virus-specific protein accumulation in each infected cell gel fraction (V) was determined using the expression: $i - Nu = V$, where i is the ^{3}H activity in an infected cell gel fraction, u is the ^{3}H activity in the corresponding uninfected cell gel fraction, N is the reduction in host protein accumulation (determined above), and Nu is the host cell background estimated for the infected cell gel fraction. Virus-specific accumulation was plotted as the difference analysis profile. Total ^{3}H activity present in each virus-specific protein peak was considered to represent the total accumulation of each virus-specific protein. In all instances the ^{3}H activities were corrected for background and ^{14}C spill into the ^{3}H channel.

RESULTS

Comparative protein electropherograms of uninfected L cells and L cells infected with *wt* VS virus. The polypeptide patterns of VS virus-infected and uninfected cells were resolved using SDS-polyacrylamide gel electrophoresis. The results (Fig. 1A) demonstrate the profiles of *wt*-infected and uninfected L cells labeled with [^{3}H]leucine from 2 to 3 h after adsorption at 31 C and shift up to 39 C. In each case accumulating polypeptides labeled with the same radioactive precursor were run on parallel gels with virion ^{14}C-labeled marker proteins to permit direct comparison of the electrophoretic profiles. In the infected cells the synthesis of host cell polypeptides was substantially reduced. In addition, there was an accumulation of virus-specific polypeptides identified by their co-migration with ^{14}C-amino acid-labeled pro-

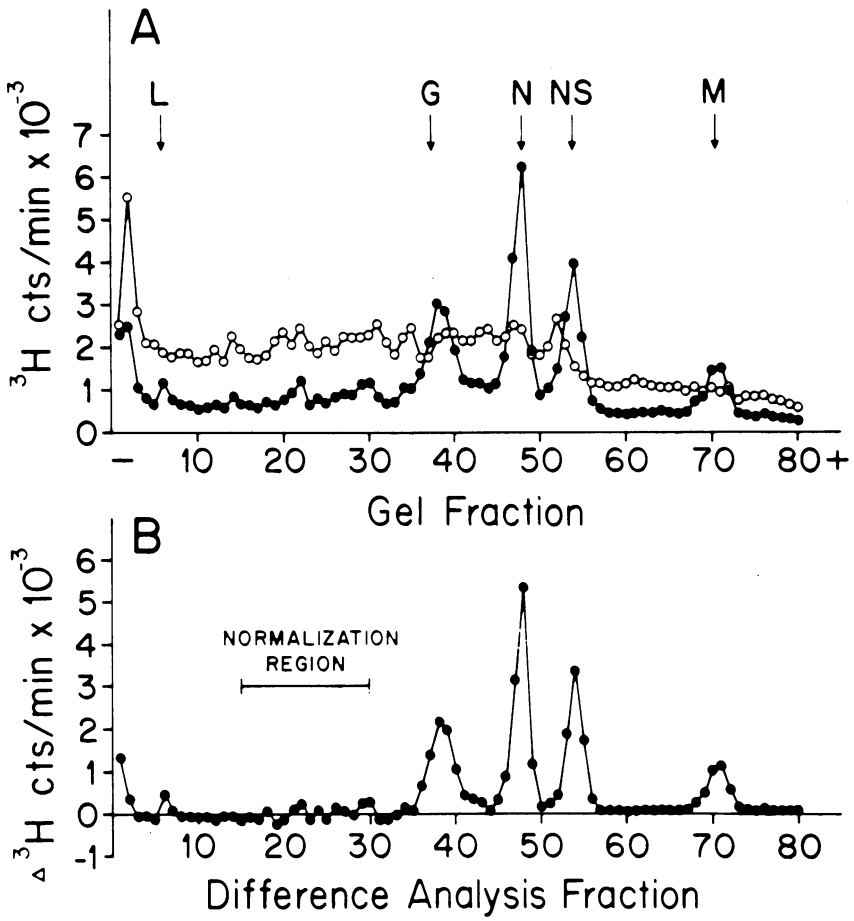

FIG. 1. *Electropherograms (A) and difference analysis (B) of proteins synthesized in uninfected L cells (○) and L cells infected with wt VS virus at MOI ≃ 100 (●). Parallel monolayer cultures of uninfected and infected L cells were incubated at 39 C, labeled from 2 to 3 h with [^{3}H]leucine (10 μCi/ml), and disrupted by treatment with 1% NP-40. The cytoplasmic contents were precipitated with 10% trichloroacetic acid suspended in 2% SDS and 1% 2-mercaptoethanol and subjected to electrophoresis in parallel on 7.5% polyacrylamide gels for 16 h at 4 mA/gel along with VS virion marker [^{14}C]proteins (marked by arrows). (B) Difference analysis profile for the virus-specific proteins determined by compensating for cellular proteins in the uninfected cell gel by the normalization ratio as described for the gel region devoid of viral proteins.*

teins of purified VS virions (arrows). The virus-specific polypeptides are designated L (large), G (glycoprotein), N (nucleocapsid), NS, and M (membrane) (24). There was no evidence in the infected cell electropherograms to indicate that any additional virus-specific proteins are found in infected cells that are not found in purified virions. This same characteristic profile was observed in *wt*-infected cells incubated at 31 C (data not shown).

Since virus-specific polypeptides are readily identified, the comparative inhibition of host cell protein synthesis and accumulation of virus-specific proteins were determined by difference analysis of virus-infected and uninfected cell electropherograms. The results of the difference analysis are illustrated in Fig. 1B. The region of each gel extending from fractions 15 to 30 was chosen to compare cellular proteins synthesized in uninfected and infected cells because in this gel region no viral proteins are evident. As described above, the total ^{3}H activity in this region of the infected cell electropherogram was divided by the total ^{3}H activity in the corresponding region of the uninfected cell electropherogram. This normalization ratio indicates directly the degree of inhibition of host cell protein accumulation. The ^{3}H-labeled protein activity of each uninfected cell fraction is then multiplied by this normalization ratio.

The value obtained, representing host cell background, is subtracted from the ^3H activity of the corresponding infected cell fraction to yield the virus-specific activity (Fig. 1B). The near base-line activity in those regions devoid of viral proteins reflects the reliability of this analytical method to determine viral inhibition of synthesis of a major group of cellular proteins.

These data also suggest an inverse relationship, although not a direct substitution, between synthesis of viral proteins and host cell proteins in L cells infected with *wt* VS virus. To validate this temporal relationship, electrophoresis and difference analyses of viral and cell proteins were examined by three 1-h labeling periods during the cycle of viral infection. Figure 2 depicts the absolute rates of accumulation of each of the five VS viral proteins compared with the decline in the absolute rate of host protein synthesis in the same infected L cells. Clearly, only very low levels of viral protein could be detected during the first 1 h of infection.

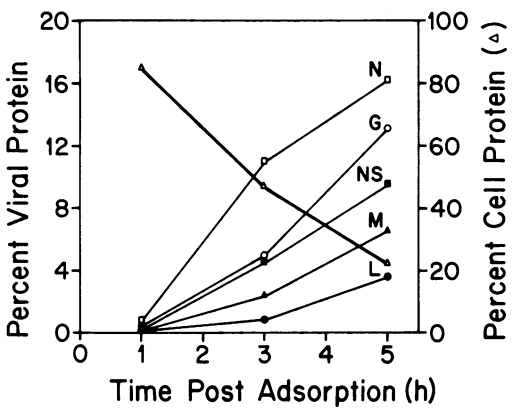

FIG. 2. *Synthesis of virus-specific proteins and inhibition of host protein synthesis in cells infected with wt VS virus. Mouse L cells, infected (multiplicity of infection ≃ 100) and incubated at 31 C, were labeled for 1 h with [^3H]leucine (10 μCi/ml) at 0 to 1, 2 to 3, and 4 to 5 h postadsorption. Cells were harvested as described, and proteins of corresponding infected and uninfected cell preparations were fractionated by electrophoresis in parallel on 7.5% SDS-polyacrylamide gels. The difference analysis profiles were calculated to indicate the radioactivity of virus-specific protein peaks. The amount of each viral protein (L, G, N, NS, and M) at each time interval is expressed as the percentage of total protein accumulation in the uninfected cell. The synthesis of cellular protein is also expressed as the percentage of total protein accumulation in the uninfected cell. Both viral and host cell protein synthesis in the infected cell is expressed as the absolute rate of synthesis.*

Subsequent labeling periods revealed progressively greater amounts of viral protein synthesis and the concomitant decline in cell protein synthesis. The absolute rates of protein synthesis clearly indicate that there is a decline in host protein synthesis that is not merely a competitive substitution by viral protein synthesis.

Characterization of RNA$^-$ *ts* mutants by their transcriptional activity in vivo. Although the VS virus RNA$^-$ mutants of complementation groups I and IV have been well characterized by transcription in vitro (11, 21) and to some extent in vivo (16, 22), a comparative analysis has not yet been made of the mRNA species transcribed by group I and group IV mutants at restrictive temperature. Detailed studies are now in progress in this laboratory of the in vivo RNA synthetic activities of those *ts* mutants of VS virus thought to be restricted in transcription and/or replication (R. R. Wagner, R. M. Snyder, P. J. Edgerton, unpublished data). To establish a firmer foundation for the present studies on synthesis of cellular and viral proteins, it seemed essential to report some of our data on the comparative in vivo transcriptive activity of representative RNA$^-$ *ts* mutants in complementation groups I and IV. To this end we analyzed by polyacrylamide gel electrophoresis the polyadenylated cytoplasmic mRNA extracted from L cells infected with *ts* mutants under permissive and restrictive conditions.

Monolayer cultures of ~2 × 10^7 L cells were infected with *wt*, *ts*114 (I), or *ts*44 (IV) VS virus at multiplicities of ~50. After adsorption at 31 C for 30 min, the cells were washed and dispersed by scraping in minimal essential medium and incubated with actinomycin D (10 μg/ml) in spinner cultures immersed in water baths at 31 ± 0.2 or 39 ± 0.2 C. The infected cells were pulse-labeled by adding [^3H]uridine to a final concentration of 50 μCi/ml. Cells were harvested by chilling, centrifugation, and washing in cold phosphate-buffered saline and then disrupted with 1% NP-40. Nuclei and other debris were removed by pelleting at 800 × *g* for 10 min. The RNA in the supernatant fluid was extracted with phenol and 0.1% SDS, the phenol phase was re-extracted, and the RNA was precipitated overnight with 2.5 volumes of ethanol at −20 C. After washing the precipitate three times with 70% ethanol, the RNA was suspended in 0.6 ml of 0.5 M NaCl and 0.05% SDS in 0.01 M Tris-hydrochloride, pH 7.4. The [^3H]RNA was then fractionated by affinity chromatography on oligo(dT) in a 1-ml syringe column equilibrated with buffered 0.5 M NaCl. Nonadenylated RNA was washed

through, and the adherent polyadenylated RNA was eluted in salt-free buffer. The polyadenylated [^{3}H]RNA species with added 28S and 18S ribosomal [^{14}C]RNA marker were analyzed by electrophoresis for 3 h at 5 mA/gel on 2.0% polyacrylamide gels.

Figure 3 compares the electropherograms of VS viral polyadenylated mRNA made 3.5 to 4 h after infection of L cells with *ts*114 (I) at 31 and 39 C and with *ts*44 (IV) at 39 C. As noted, comparable amounts of 30S and 13-18S viral mRNA species were made in L cells infected with *ts*114 (I) at 31 C and with *ts*44 (IV) at the restrictive temperature of 39 C. Comparable mRNA species were found to hybridize completely to VS virion RNA (data not shown). In sharp contrast, no viral RNA could be detected in a parallel culture of L cells infected with *ts*114 (I) at 39 C (Fig. 3). Hybridization data also indicate that *ts*114 is completely restricted in in vivo primary transcription at 39 C (D. H. L. Bishop, personal communication). Identical 30S and 12-18S mRNA species were also synthesized in L cells infected at 31 C with *ts*44 (IV) or with *wt* virus at 31 C or 39 C, but levels of mRNA were somewhat greater, as expected for amplified secondary as well as primary transcription (data not shown).

These results confirm and extend the observations that transcription of the group I mutant *ts*114 is completely restricted in vivo, whereas the *ts*44 mutant in complementation group IV is capable of transcribing at least one round of mRNA under conditions which restrict total viral RNA synthesis.

Inhibition of host cell protein synthesis after infection with *ts* mutants of VS virus. Difference analysis was applied to polyacrylamide electropherograms to determine the effect of infection with *ts* mutants on L cell protein synthesis at permissive and restrictive temperatures. Transcriptase-restricted mu-

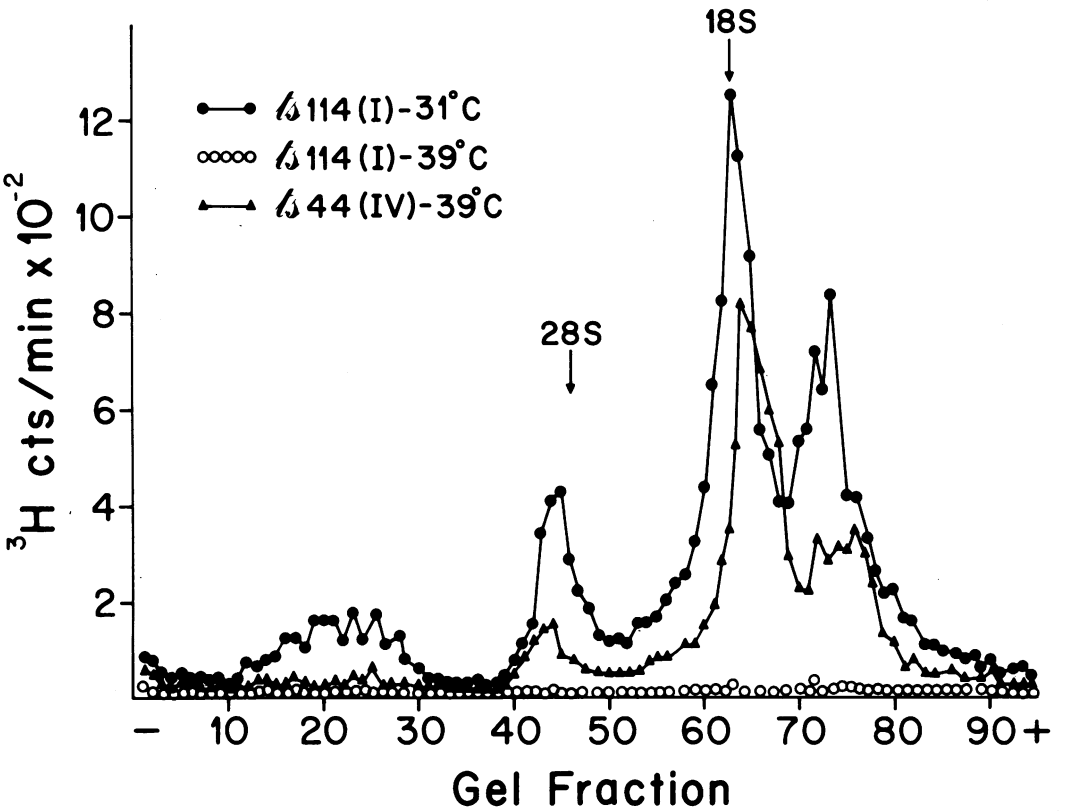

FIG. 3. *Polyacrylamide gel electrophoresis of VS viral mRNA synthesized in L cells infected with ts114 (I) at 31 and 39 C and with ts44 (IV) at 39 C. Suspension cultures of infected L cells in the presence of actinomycin D were pulse-labeled with [^{3}H]uridine (50 μCi/ml) at 3.5 to 4 h postinfection. As described in the text, RNA was extracted from infected cells with phenol and SDS and fractionated on an oligo(dT) column. Polyadenylated mRNA was subjected to electrophoresis for 3 h at 5 mA/gel on 2.0% polyacrylamide gels along with marker rRNA (arrows).*

tants of complementation group I (*ts*114 and *ts*13) were compared with the transcriptase-competent RNA$^-$ mutant of group IV (*ts*44) as well as *wt* virus and the Orsay mutant of complementation group II (*ts*52), which exhibits only slight and variable restriction in RNA synthesis (RNA$^\pm$) (5). In each case electropherograms of cells infected with each of these *ts* mutants at permissive temperature (31 C) revealed protein patterns and difference analysis profiles characteristic of cells infected with *wt* virus (see Fig. 1). L cells infected at restrictive temperature (39 C) with certain *ts* mutants exhibited distinctly different protein patterns and difference analysis profiles.

Figure 4 shows the electropherogram and dif-

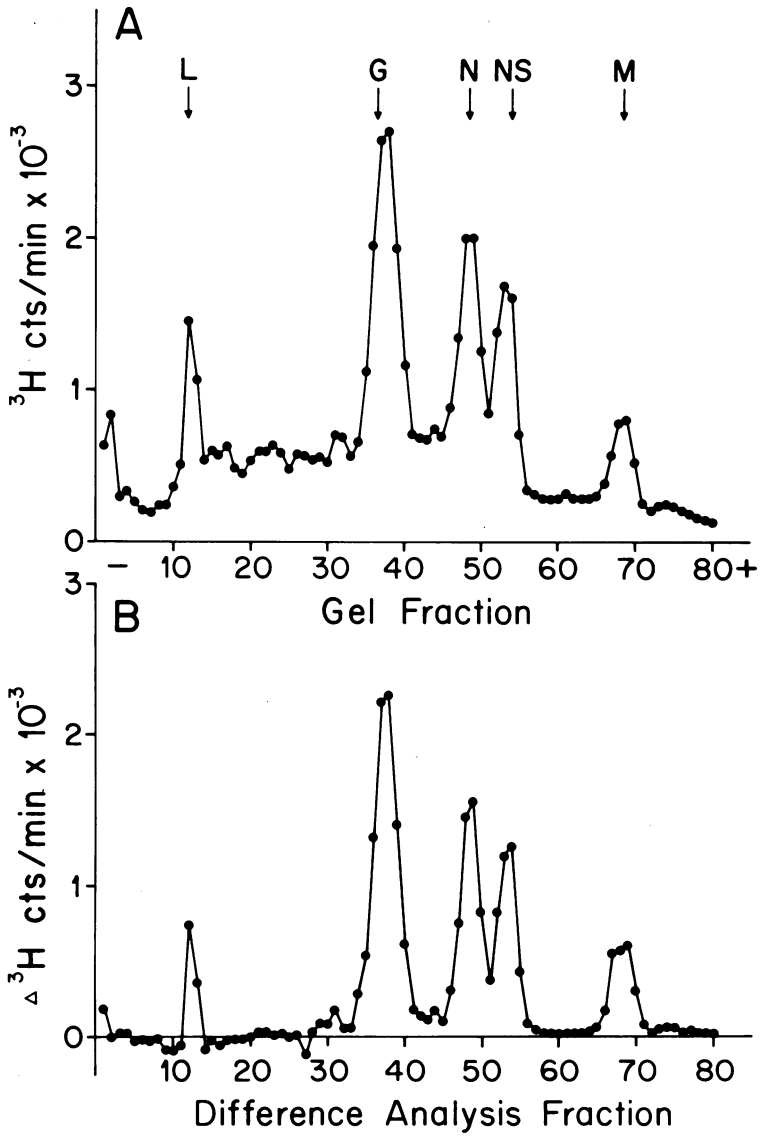

FIG. 4. *Electropherogram of VS viral and cellular proteins in L cells infected with ts44 at 39 C (A) and difference analysis profile of viral protein species in infected cells calculated by correcting for cellular proteins present in a parallel uninfected culture of L cells analyzed by gel electrophoresis (B). L cells were infected with ts44 (multiplicity of infection = 100), incubated at 39 C, and labeled with [^{3}H]leucine (10 μCi/ml) 4 to 5 h postadsorption. Proteins of infected and uninfected cells extracted after incubation for 5 h were subjected to electrophoresis in 7.5% SDS-polyacrylamide gels for 16 h at 4 mA/gel along with VS virion marker ^{14}C-labeled proteins (arrows).*

ference analysis of L cells labeled with [^{3}H]leucine 4 to 5 h after infection at 39 C with *ts*44 (IV). As noted, all five VS viral proteins are readily identifiable, but the level of viral protein N is considerably reduced, whereas that of protein G is significantly increased. A similar result using group IV mutants has previously been reported (19). The protein and difference analysis profiles (Fig. 5A and 5B, respectively) of cells infected with *ts*114 indicate that no viral proteins are synthesized at the restrictive temperature. This same result was observed with cells infected with *ts*13 (data not shown). This finding is in accord with the observation that group I mutants are transcriptionally defective at the restrictive temperature (11, 16, 17; Fig. 3).

The effect of *ts* mutants on host cell protein synthesis was determined by comparing infected and uninfected cell protein profiles over the normalization region (gel fractions 15 to 30). The ratio of viral protein-free radioactivity in infected cells divided by that in uninfected cells indicates the degree to which synthesis and accumulation of host cell proteins are inhibited. Table 1 summarizes the level of cell protein accumulation observed at different times after infection with *wt* and mutant viruses at the

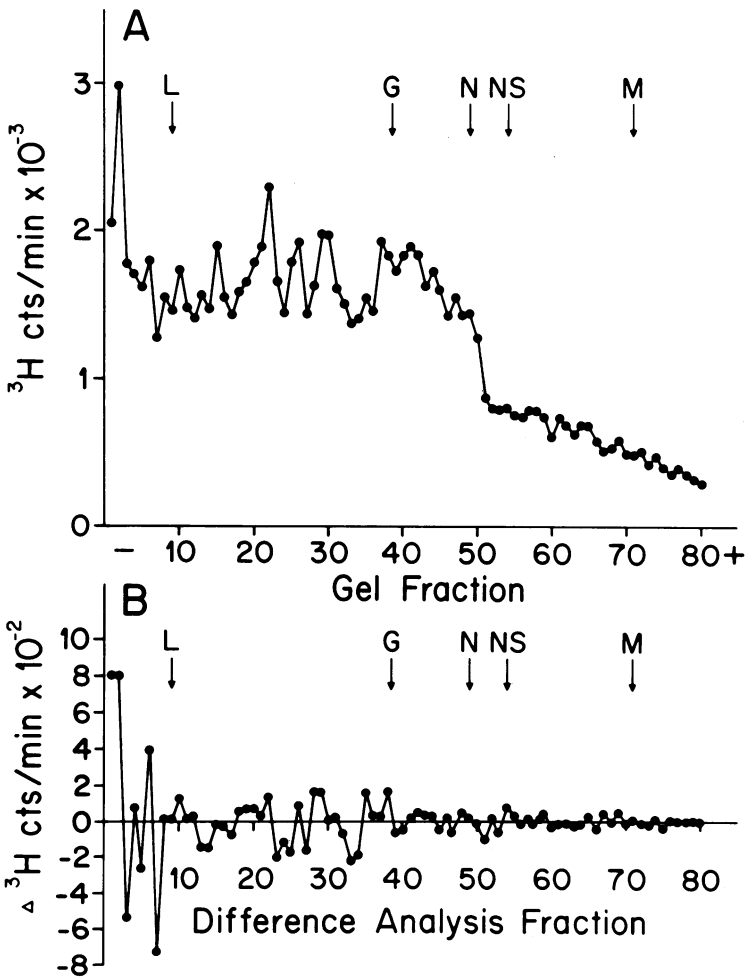

FIG. 5. *Electropherogram of proteins present in L cells infected with ts114(I) at 39 C (A) and difference analysis profile (B) of viral proteins in infected cells determined by compensation for cellular proteins in a parallel culture of uninfected cells also incubated at 39 C. Each culture was labeled with [^{3}H]leucine (10 μCi/ml) 4 to 5 h postadsorption, and the proteins were analyzed by polyacrylamide gel electrophoresis as described in the legend for Fig. 4 and in Materials and Methods. The arrows mark the positions of co-electrophoresed VS virion marker ^{14}C-labeled proteins.*

TABLE 1. *Host cell proteins synthesized at permissive (31 C) and restrictive (39 C) temperatures in L cells pulse-labeled with [³H]leucine at intervals after infection with wt and ts mutants of VS virus*

Incubation temp (C)	Time after adsorption (h)	[³H]leucine-labeled nonviral proteins in infected cells (% uninfected cell proteins)[a]				
		wt	*ts*114 (I)	*ts*13 (I)	*ts*44 (IV)	*ts*52 (II)
31	0-1	85	96	93	101	91
	2-3	47	65	72	41	77
	4-5	22	23	38	9	35
39	0-1	72	105	87	92	86
	2-3	40	99	90	50	68
	4-5	13	101	96	29	42

[a] Host cell protein synthesis in L cells infected with *wt* and *ts* mutants was determined by comparative analysis of radioactivity in regions of polyacrylamide gels devoid of VS viral proteins. The calculations for percentage of host protein in infected cells were made by difference analysis with comparable uninfected cells at the same period of labeling as described.

permissive and restrictive temperatures. The cellular protein accumulation observed in virus-infected cells is expressed as a percentage of that observed in the uninfected cells. At both the permissive and restrictive temperatures, host protein synthesis was significantly and progressively reduced in cells infected with *wt*, *ts*44, or *ts*52. This same pattern of cell protein reduction was exhibited in cells infected with *ts*114 or with *ts*13 at the permissive temperature. At the restrictive temperature, however, no reduction in host protein synthesis was observed in cells infected with either of the group I mutants, *ts*114 and *ts*13.

These results clearly indicate that viral RNA transcription is required for the reduction of host cell protein synthesis. Due to the high multiplicity of infection (100), the residual, uninhibited synthesis of host cell protein is probably due to incomplete inhibition rather than uninfected cells.

DISCUSSION

The salient feature of these experiments would appear to be that transcriptase-defective group I mutants of VS virus fail to switch off cellular protein synthesis under restrictive conditions, whereas a transcriptase-competent group IV RNA⁻ mutant (11, 21) effectively inhibits cell protein synthesis. Possibly related to this differential effect on cellular protein synthesis are correlative data that avirulent group I mutants are restricted in primary transcription and translation, in contrast to the competence of the group IV mutant to carry out primary transcription and translation (2, 20). The relatively efficient switch off by *ts*44 (IV) of cellular protein synthesis would appear to rule out dependence on replication or other events subsequent to primary transcription and translation. Since no mutants appear to be restricted at the level of translation, it is not possible to pose the question whether viral transcription alone is sufficient to block protein synthesis or other cellular functions.

The failure of transcriptase-defective mutants of complementation group I to switch off protein synthesis at restrictive temperatures and at high input multiplicity appears to rule out an earlier hypothesis (10, 28) that nonreplicating toxic components of the input virion are responsible for inhibiting macromolecular synthesis and causing cell death. The results reported here clearly indicate that a transcriptional function is the minimal synthetic event required for inhibiting cell protein synthesis, as demonstrated by the action of *ts*114 (I). Less direct data support the hypothesis that VS viral transcription must precede inhibition of cell function. Marcus and Sekellick (12, 13) have clearly shown that intact function of the virion-associated polymerase is essential for cell killing. There is also suggestive evidence that defective-interfering long T virions of VS virus interfere with cytocidal infection by blocking transcription of B virions (9).

The nature of the cytocidal component of VS virus is not readily discernible, but the only obvious candidates are newly synthesized viral RNA and proteins. Recent experiments with *ts* mutants seem to suggest that primary transcripts alone are inefficient in cell killing (13). Other studies have shown that de novo protein synthesis is required for VS virus to manifest a cellular effect (27). However, earlier studies revealed that cellular RNA synthesis can be inhibited by VS virus even though viral protein synthesis is blocked by interferon (28). These contradictory findings led to an hypothesis that VS virus alters cell functions in two distinct ways: (i) an early multiplicity-dependent and UV-insensitive event (10, 28), and (ii) a progressive, UV-sensitive inhibition (28). Our results appear to cast doubt on the validity of the first hypothesis.

Post-transcriptional RNA species would not appear to be the most likely candidates for the VS viral cytocidal product(s). Although *ts*44 synthesizes typical 30*S* and 13-18*S* viral mRNA at restrictive temperatures, there is reasonable evidence that progeny 40*S* RNA synthesis is suppressed (18, 19). However, it is not possible to rule out completely the occurrence of certain

replicative events even when group IV mutants are restricted; a complicated interdependent switching from transcription and replication has been postulated for the transcriptase-restricted group I mutant *ts*114 (14, 15). Whether such events could result in synthesis of double-stranded viral RNA postulated as a possible cell-killing factor for VS virus (13) and other viruses (1, 6) remains to be examined in more detail.

The results reported here strongly suggest that complete viral replication is not required for manifestation of an inhibitory effect on cell protein synthesis, but viral transcription and possibly translation appear to be essential.

ACKNOWLEDGMENTS

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