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Two Transcription Products of the Vesicular Stomatitis Virus Genome May Control L-Cell Protein Synthesis

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When mouse L-cells are infected with vesicular stomatitis virus, there is a decrease in the rate of protein synthesis ranging from 20 to 85% of that in mock-infected cells. Vesicular stomatitis virus, irradiated with increasing doses of UV light, eventually loses this capacity to inhibit protein synthesis. The UV inactivation curve was biphasic, suggesting that transcription of two regions of the viral genome is necessary for the virus to become inactivated in this capacity. The first transcription product corresponded to about 373 nucleotides, and the second corresponded to about 42 nucleotides. Inhibition of transcription of the larger product by irradiating the virus with low doses of UV light left a residual inhibition of protein synthesis consisting of approximately 60 to 65% of the total inhibition. This residual inhibition could be obviated by irradiating the virus with a UV dose of greater than 20,000 ergs/mm^2 and was thus considered to represent the effect of the smaller transcription product. In the R1 mutant of C. P. Stanners et al. (Cell 11:273–281, 1977), inhibition of transcription of the larger product sufficed to restore protein synthesis to the mock-infected level, suggesting that the smaller transcription product is nonfunctional with respect to protein synthesis inhibition. It thus appears that the inhibition of protein synthesis by wild-type vesicular stomatitis virus involved at least two separate viral transcription products, and the inhibition by the R1 mutant involved only one. Extracts from cells infected with virus irradiated with low doses of UV light showed a protein synthesis capacity quite similar to that of their in vivo counterparts, indicating that these extracts closely reflect the in vivo effects of virus infection.

Vesicular stomatitis virus (VSV) has been shown to inhibit protein synthesis in suitable host cells (4, 10, 13, 16, 18, 24, 29). The kinetics and extent of inhibition are somewhat dependent on the cell line and virus strain (14, 24). As can be seen by analysis of the products by polyacrylamide gel electrophoresis, the cellular proteins are inhibited, whereas the viral proteins flourish. Stanners et al. (24) and Jaye et al. (10) have shown that protein synthesis inhibition occurs at the level of polypeptide chain initiation. Furthermore, Centrella and Lucas-Lenard (4) have observed that virus infection causes a decrease in initiation factor 2 activity in extracts from infected cells.

Lodish and Porter (13, 14) have suggested that the inhibition of cellular protein synthesis results from competition between viral and cellular mRNAs for existing ribosomes. This rationale follows if one considers that with viral infection, there is a two- to threefold increase in total translatable mRNA within 4 h of infection (10, 13, 14). This large increase in total mRNA results from the extremely efficient transcription of the viral genome.

Other data, however, suggest that specific viral products other than viral mRNAs are involved in the inhibition (16–18, 24). Stanners et al. (24) have called the virus-specific product involved in the inhibition the P function, and they have isolated and partially characterized a P$^-$ mutant (R1) that has a lower capacity to inhibit total protein synthesis than does its parent wild type. Using UV light-inactivated virus, Marvaldi et al. (17) have found that the inhibition of protein synthesis correlates with the expression of the N protein gene. Such UV-irradiated virus is incapable of secondary transcription, and thus the total number of transcripts in cells infected with this virus is greatly reduced (6).

In an effort to understand the inhibition phenomenon, we chose the UV light inactivation technique to construct viruses that express the inhibitory factor(s), yet do not produce significant amounts of viral mRNA. This technique

can be used because viral mRNA transcription is sequential (1, 2) and therefore allows a successive inhibition of viral mRNA transcription (the most UV-resistant transcript being that nearest the transcription initiation site and the least UV-resistant being that furthest from that site). Our results show that the UV-inactivation curve was biphasic with the HR strain of the Indiana serotype of VSV and monophasic with the R1 mutant characterized by Stanners et al. (24). These results suggest that protein synthesis inhibition by the HR strain involves at least two viral transcription products and that the R1 mutant probably lacks one of these.

Finally, we prepared cell-free extracts from the UV-irradiated, virus-infected cells and found that the protein synthesis capacity of these extracts reflected quite closely that of their in vivo counterparts. Therefore, these extracts will enable us to dissect the molecular mechanism of protein synthesis inhibition by VSV.

MATERIALS AND METHODS

Cells and virus. Monolayers of mouse L-cells were grown and maintained in Richter minimal essential medium (Irvine Scientific Co.) plus 5% bovine calf serum on plastic petri dishes at 37°C.

A heat-resistant strain of the Indiana serotype (HR-C) of VSV was used throughout this study (8). The preparation, storage, and analysis of this virus have been described previously (10). The titer of this virus stock was typically found to be $1.10 \times 10^{11} \pm 0.25 \times 10^{11}$ PFU/ml. The R1 mutant used in this study was isolated by Stanners et al. (24). It is a revertant of the double mutant T1026 and was generously supplied to P. I. Marcus at the University of Connecticut by C. P. Stanners. The R1 mutant was grown on Vero-C1 cells, and the supernatant from these cells was collected at 18 h after infection. The clarified supernatant (10,000 $\times$ *g* for 10 min) was used as stock virus. Its titer was typically 10^9 PFU/ml.

Infection of L-cells was essentially the same as described previously by Wu and Lucas-Lenard (30). Adsorption was for 45 min at 37°C.

UV irradiation of virus. The virus stock was diluted (ca. 1/1,000) with phosphate-buffered saline to a concentration that would allow delivery of the desired multiplicity of infection (MOI). The diluted virus was transferred to a prewetted Falcon dish and kept on ice. The virus was then irradiated with UV (average wavelength [$\bar{\lambda}$], 254 nm) light from a General Electric germicidal bulb. The lamp was calibrated against a Blak-Ray ultraviolet meter (J-22S shortwave UV meter; Ultraviolet Products, Inc.). At 90.5 cm from the sample, the output of the lamp was 7.3 ± 1.0 ergs/mm^2 per s; at 39.5 cm, the output was 26.0 ± 1.0 ergs/mm^2 per s. The sample was then transferred to a sterile plastic tube and kept on ice until used for infection.

Isotope labeling. Double-isotope labeling was performed essentially as described previously by Marvaldi et al. (17). L-cells were prelabeled with 33.3 μCi of ^{3}H-amino acids mixture (NET-250; New England Nuclear Corp.) per ml in Richter minimal essential medium plus 5% bovine calf serum for 2 to 4 h and pulse-labeled with 3 to 5 μCi of L-[^{35}S]methionine (specific activity, >1,000 Ci/mmol; NEG-009T; New England Nuclear Corp.) in Richter minimal essential medium plus 5% bovine calf serum for 30 min at the times indicated in the figure legends and table footnotes. The infection was quenched by placing the cells on ice. The cells were lysed either with 0.4% Triton X-100 in RSB (as defined in Jaye et al. [10]) or with 0.1 N NaOH. The Triton-treated lysates were transferred to 1.5-ml micro test tubes and centrifuged at 13,000 $\times$ *g* for 5 min. The soluble fractions from the Triton- or NaOH-treated lysates were neutralized, and then trichloroacetic acid was added to a final concentration of 5%. The Triton-extracted material was heated at 90°C for 5 min to deacylate the charged tRNA. The trichloroacetic acid precipitates from the Triton- or NaOH-treated lysates were filtered onto Whatman GF/C filters and washed with 5 volumes of 5% trichloroacetic acid followed by 5 volumes of 95% ethanol. The dried filters were analyzed in toluene/2a70 (Research Products International Corp.) scintillation cocktail in a Tracor Mark III scintillation counter programmed for double-label counting. Repeated experiments have consistently allowed for determinations of ±10% or better.

Cell-free systems. The protocol for the preparation of the extracts was essentially that described by Jaye et al. (10). Briefly, L-cells on 100-mm petri dishes were given fresh medium and serum for 2 h before infection. The cells were infected with unirradiated or irradiated virus (MOI, 18 PFU per cell). The infection was stopped by placing the plates on ice. The cells were then gently scraped from the plates with a rubber policeman. The cells were washed twice with ice-cold Richter minimal essential medium and centrifuged. The pelleted cells were suspended in 1.5 volumes of hypotonic buffer (10) for 8 min on ice and then lysed in a Dounce homogenizer. The lysate was centrifuged at 22,000 $\times$ *g* at 4°C for 15 min. The supernatant fraction (S-22) was removed and adjusted with 10 times concentrated HKMD, as described previously by Jaye et al. (10). The conditions for cell-free incubations were as described previously (10). The optimal Mg^{2+} concentration for amino acid incorporation was 3.0 mM with extracts from both mock-infected cells and cells infected with unirradiated (UV = 0 ergs/mm^2) virus. The extracts from cells infected with virus that had been irradiated at doses of 330 or 2,200 ergs/mm^2 had broader Mg^{2+} optima (3 to 6 mM). The Mg^{2+} concentration used in each incubation was 3.0 mM, and the ATP concentration was 0.9 mM. The amount of protein in each lysate was determined as described previously by Bradford (3). In each incubation, 2 to 5 μCi of L-[^{35}S]methionine (>1,000 Ci/mmol) was present.

The counts per minute of [^{35}S]methionine incorporated into hot trichloroacetic acid-precipitable material during 15 min of incubation at 32°C in a 50-μl reaction mixture was used as a measure of protein synthesis. The amount of methionine incorporated in terms of picomoles was calculated by converting counts per minute to disintegrations per minute and dividing the disintegrations per minute by the specific radioactivity of the methionine. The specific radioactivity of the methionine was determined by measuring the L-methionine pool size for each lysate, using the isotope dilution technique. The values for the respective ly-

sates were: mock infected, 34 μM; VSV (UV = 0 ergs/mm^2), 33 μM; VSV (UV = 330 ergs/mm^2), 44 μM; and VSV (UV = 2,200 ergs/mm^2), 33 μM. Thus, picomoles of methionine incorporated = disintegrations per minute/(curies of radioactive methionine added/total methionine pool size).

Polyacrylamide gel electrophoresis. The samples and gels were prepared essentially as described previously (4), except that the gels were poured as linear gradients from 7.5 to 15% acrylamide. X-Omat AR film (Eastman Kodak Co.) was used for autoradiography.

Target size analysis. Target size analysis has been described elsewhere (12). Briefly, the general expression for one-hit multitarget kinetics is $N/N_0 = me^{-rF}$, where N_0 is the number of targets present before irradiation, N is the number of surviving targets, F is the radiation dose (ergs per square millimeter), r is the cross section for inactivation, or rather, the target size (square millimeters per ergs), and m is the number of targets which must be hit to cause an inactivation event. Thus, the "surviving fraction" is N/N_0. In the actual experiment for protein synthesis inhibition, the surviving fraction was computed as N = (rate of synthesis in mock-infected cells − rate in cells infected with irradiated virus)/rate in mock-infected cells and N_0 = (rate of synthesis in mock-infected cells − rate in cells infected with irradiated virus)/rate in mock-infected cells.

Infectivity analysis was used as a calibration of the rate of inactivation, since one hit to any part of the genome is necessary and sufficient to inactivate replication. The size of the VSV (HR-C) genome was assumed to be 3.6×10^6 daltons (27). The slope of the curve for survival of protein synthesis inhibition as a function of UV dose, relative to the slope of the curve for survival of infectivity, was used to calculate the fractional amount of the genome required to inhibit protein synthesis.

RESULTS

The effect of UV-irradiation on the ability of VSV to inhibit protein synthesis. Using UV irradiation to inactivate the viral genome, Marvaldi et al. (17) showed that there is a correlation between protein synthesis inhibition and expression of the N protein gene. To obtain a better estimate of the target size necessary for protein synthesis inhibition, we UV irradiated the virus at different doses and then measured the ability of those irradiated virus preparations to inhibit protein synthesis. The protein synthesis rate was determined by pulse-labeling cells for 30 min with [^{35}S]methionine and measuring [^{35}S]methionine incorporation into hot trichloroacetic acid-insoluble material. In L-cells infected with VSV that had been irradiated with doses of up to 550 ergs/mm^2, there was an apparent increase in the inhibition of protein synthesis (Fig. 1A, upper curve). Analysis of the radiolabeled products from this experiment by polyacrylamide gel electrophoresis (Fig. 1B) revealed that the increase was, in fact, a result of a decrease in viral protein synthesis. The gel analysis also showed that at a dose between 440 and 550 ergs/mm^2 (lanes E and F), the point of maximal protein synthesis inhibition, the N protein was the only viral protein detected. It is most important to note that cellular protein synthesis was severely inhibited after infection even when viral protein synthesis (and presumably viral mRNA synthesis) was drastically reduced.

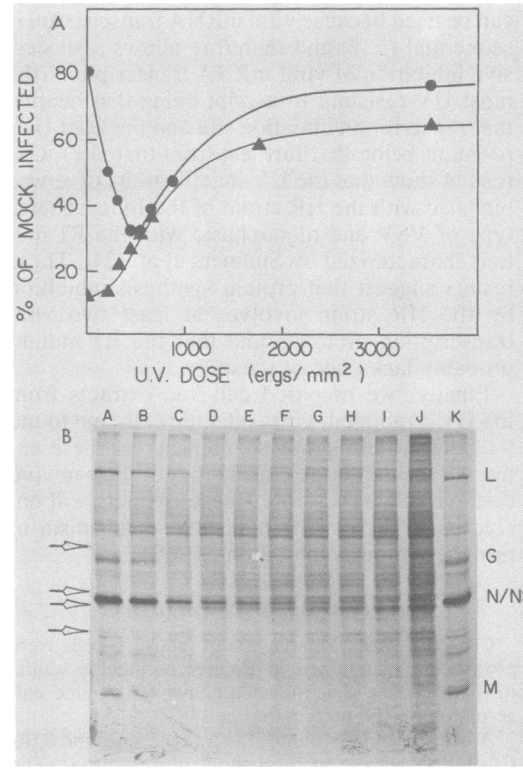

FIG. 1. Effect of UV-inactivated VSV on L-cell protein synthesis. L-cells were prelabeled with L-^{3}H-amino acids for 2 h in fresh medium and serum. The cells were infected at an MOI of 50 PFU per cell with VSV that had been irradiated to the indicated degree or were mock infected with phosphate-buffered saline. The infected monolayer (●) or suspension culture (▲) of L-cells was incubated for 3.0 or 5.0 h, respectively, at 37°C, then pulse-labeled for 0.5 h at 37°C with L-[^{35}S]methionine in complete medium, except for methionine, which was present at 10 μM, plus 5% dialyzed bovine calf serum. The infection was quenched, and a lysate was prepared with Triton, as described in the text. (A) Inhibition of protein synthesis by UV-irradiated virus. (B) Autoradiogram from a sodium dodecyl sulfate-gel electrophoresis analysis in which 100 μg of protein per lane was loaded: (A) unirradiated, (B) 100 ergs/mm^2, (C) 220 ergs/mm^2, (D) 330 ergs/mm^2, (E) 440 ergs/mm^2, (F) 550 ergs/mm^2, (G) 660 ergs/mm^2, (H) 1,100 ergs/mm^2, (I) 2,200 ergs/mm^2, (J) mock infected, (K) unirradiated. L, L protein; G, G protein; N/NS, N and NS proteins; M, M protein. Arrows, L-cell proteins reappearing at high UV doses (see text).

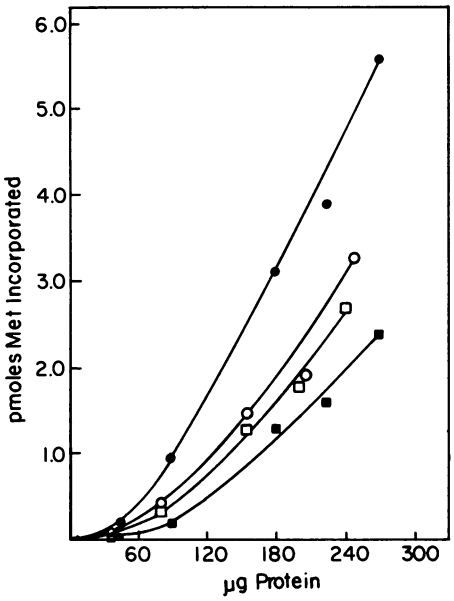

FIG. 2. Analysis of extracts prepared from L-cells infected with UV-irradiated VSV. The preparation, incubation conditions, and analysis of the cell-free systems are described in the text. Symbols: ●, mock infected; ○, VSV (UV = 0 ergs/mm^2); ■, VSV (UV = 330 ergs/mm^2); □, VSV (UV = 2,200 ergs/mm^2). Met, Methionine.

Infecton of L-cells with VSV irradiated at doses greater than 550 ergs/mm^2 resulted in a decreasing degree of protein synthesis inhibition (Fig. 1A), and, as shown by the arrows in Fig. 1B, several host proteins began to reappear. Interestingly, even up to a dose of 3,500 ergs/mm^2 (Fig. 1A), there was a detectable amount of protein synthesis inhibition. This residual amount varied from experiment to experiment and ranged from a minimum of 15% to a maximum of 50%.

On some occasions (2 of 10 experiments) after infection of cells in monolayers or suspension culture, the UV inactivation curve looked somewhat different; the initial point (0 ergs/mm^2) represented the maximal amount of inhibition, and after UV irradiation, the inhibition was less and less (see lower curve in Fig. 1A). We have no satisfactory explanation for this observation at the moment, and studies designed to clarify this phenomenon are under way in our laboratory.

Comparison of [^{35}S]methionine incorporation into protein in whole cells and in cell-free extracts. In these studies, we examined the extent to which extracts from infected cells reflected the effects of virus infection in vivo. We based these studies on the observation that when VSV is irradiated with different doses of UV light, protein synthesis is inhibited to various extents. We rationalized that extracts prepared from cells infected with irradiated virus should reflect the in vivo protein synthesis activity of the cells.

L-cells in monolayer cultures were mock infected or infected with unirradiated or irradiated virus, and 3.75 h after infection some of the cultures were harvested and lysed as described above. The remaining cultures were pulse-labeled with [^{35}S]methionine in vivo at the same time, so that in vitro and in vivo rates of protein synthesis could be compared.

The amino acid pool size was estimated for each extract (mock infected, UV = 0, 330, or 2,200 ergs/mm^2) by isotope dilution techniques, and the incorporation data were corrected for differences in pool sizes, as discussed above. Incorporation of L-[^{35}S]methionine was maximal in the extract from mock-infected cells and minimal in the extract from cells infected with virus irradiated at a dose of 330 ergs/mm^2 (Fig. 2). The extract from cells infected with unirradiated virus was less active than the mock-infected extract, but more active than the extracts from cells infected with virus irradiated at a dose of 330 or 2,200 ergs/mm^2. Hence, the cell-free lysates reflected to the whole cell experiments demonstrated in Fig. 1A in the ability of the lysates to incorporate L-[^{35}S]methionine.

The products of the in vitro radiolabel incorporation experiments were examined by polyacrylamide gel electrophoresis and were compared with the products labeled in vivo. There was a good qualitative correlation between the in vitro- and the in vivo-labeled products (Fig. 3). (These gels cannot be viewed as quantitative indicators of protein synthesis, since the samples labeled in vitro contained slightly different amounts of protein.) Fig. 3 also shows the presence of two apparently virus-specific bands migrating below the M protein in the gels of both in vivo- and in vitro-labeled proteins. The origin of the bands is unknown, and they disappeared after UV irradiation of the virus. Also, they were not always detected (Fig. 1B). Currently, we are trying to characterize these bands. When initiator [^{35}S]methionyl tRNA in the presence of 100 µM L-methionine was used in place of L-[^{35}S]methionine to label proteins in extracts, similar results were obtained with respect to relative incorporation and labeled products made (data not shown). Thus, both elongation and initiation were taking place in these extracts.

Quantitatively, when the percentage of protein synthesis (protein synthesis in infected cells/protein synthesis in mock-infected cells) was compared, there was a fair correlation between the in vivo and in vitro labeling experiments, as shown in Table 1. The lysates from cells infected with virus which had been irradiat-

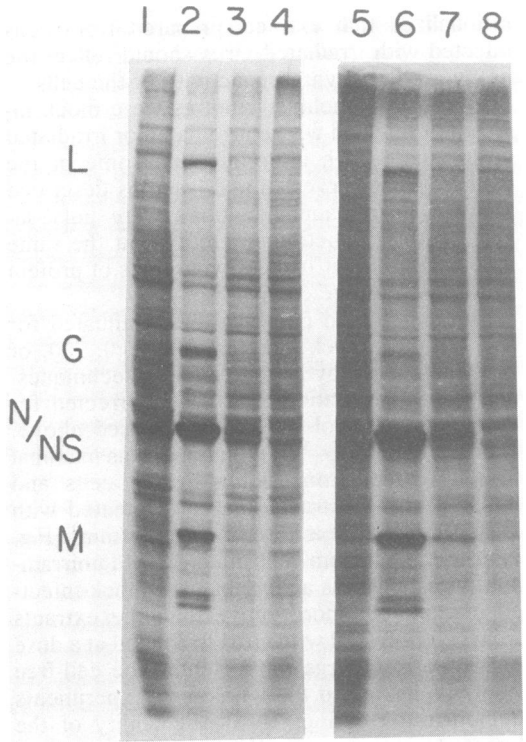

FIG. 3. Comparison of the gel electrophoretic patterns of proteins from mock-infected or VSV-infected L-cells labeled in vivo or in vitro. This is an autoradiogram of an electrophoretic analysis on a 7.5 to 15% polyacrylamide gel. Lanes 1 through 4, Cells pulse-labeled in vivo at 3.5 to 4.0 h after infection and lysed with Triton; 100 μg protein was added in each lane. Lanes 5 through 8, Cell-free incubations described in the text and depicted in Fig. 2: lane 5, 126 μg of protein; lane 6, 175 μg of protein; lane 7, 159 μg of protein; and lane 8, 170 μg of protein. Lanes 1 and 5, mock infected; lanes 2 and 6, unirradiated virus; lanes 3 and 7, virus irradiated at 330 ergs/mm^2; and lanes 4 and 8, virus irradiated at 2,200 ergs/mm^2. The autoradiogram of the proteins labeled in vitro (lanes 5 through 8) was exposed for 10 days, whereas that of the proteins labeled in vivo (lanes 1 through 4) was exposed for 4 days. L, L protein; G, G protein; N/NS, N and NS proteins; M, M protein.

ed at a dose of 330 or 2,200 ergs/mm^2 were slightly less active than their in vivo counterparts.

Analysis of small viral transcription product necessary for protein synthesis inhibition. Cellular protein synthesis was still inhibited from 15 to 40% at UV doses in which transcription would have been reduced to 10^{-4} of that with unirradiated virus (Fig. 1A and B; Table 1 and Fig. 4). This latter value was determined by a plaque reduction assay at a UV dose of 440 ergs/mm^2, and the calculation was based on the assumption that one hit anywhere in the viral

TABLE 1. Comparison of in vivo with in vitro protein synthesis activities of UV-irradiated virus-infected cells[a]

UV dose (ergs/mm^2)	% of mock-infected cell activity	
	In vivo	In vitro
0	66	63
330	53	42
2,200	65	53

[a] L-cells were infected as described in the text. For the in vivo values, two plates for each condition were pulse-labeled from 3.5 to 4.0 h after infection with [^{35}S]methionine in complete medium, except for methionine, which was present at 10 μM, and 5% dialyzed calf serum. These cells were extracted with Triton and prepared as described in the text. Protein quantity was determined for each extract, and the incorporation was computed as counts per minute per microgram of protein. Values from the in vitro assay were extracted from the linear region of the curves from Fig. 2. Values shown were computed from: picomoles of methionine incorporated per 200 μg of protein.

genome would inhibit replication and therefore secondary transcription. These results suggested that protein synthesis inhibition involved still another viral component that was less sensitive or insensitive to UV irradiation.

The characteristics of this second component were examined first by determining the time necessary for expression of protein synthesis inhibition. If virion proteins would suffice, then one might expect to see inhibition soon after virus adsorption. In cells that were infected by virus that had received a UV light dose of 4,400 ergs/mm^2, there was little inhibition until 2.25 h after infection, at which time a 33% inhibition was observed (Table 2). At 24 h after infection the inhibition was 40%, suggesting that the effect was stable. Since the inhibition was time dependent, it seemed probable that transcription of the viral genome was necessary. This suggestion was supported by the observation that when the virus was heated to 50°C under conditions in which virion-associated transcriptase activity was lost (15), the virus was no longer capable of inhibiting cellular protein synthesis (data not shown). Also, when the virus was subjected to very large doses of UV irradiation (>18,000 ergs/mm^2), it lost its capacity to inhibit cellular protein synthesis (data not shown). The product inactivated at this high UV dose will henceforth be referred to as the small transcription product. The other viral product that was inactivated by UV doses of up to 1,000 ergs/mm^2 will be called the large transcription product.

Analysis of a P$^-$ mutant. We wished to determine if the R1 mutant of Stanners et al. (24)

TABLE 2. Rate and multiplicity dependence of appearance of small transcription product

Time after adsorption, (h)[a]	% of mock-infected cell activity[b] at the following dose for MOI (PFU/cell)[c] of:			
	4,400 ergs/mm² UV		0 ergs/mm² UV	
	2.1	21	2.1	21
0.25	90	98	ND[d]	ND
1.25	93	93	ND	ND
2.25	77	77	61	59
24	ND	60		

[a] L-cell monolayers were infected or mock infected as described in the text. The cells were pulse-labeled for 30 min at the indicated times with ^{3}H-amino acids in complete medium plus 5% dialyzed calf serum.

[b] The cells were extracted with Triton, as described in the text, the protein quantity was determined for each sample, and the incorporation was computed as counts per minute per microgram of protein.

[c] Stock virus was diluted to yield an MOI of 21. Then after irradiation, a portion was diluted 1/10 for an MOI of 2.1.

[d] ND, Not done.

showed the same UV inactivation curve for protein synthesis inhibition as the wild type. Subconfluent monolayers of L-cells were infected with UV-irradiated R1 mutant at three different MOIs. The data (Fig. 4) showed the presence of a UV-sensitive region analogous to that of the wild-type virus. However, inactivation of the region of the genome specifying the large transcription product resulted in a virus that was unable to inhibit protein synthesis, suggesting that the small transcription product was not inhibitory in this mutant. The inhibition was found to be multiplicity dependent. The lower curve on this graph shows the effect of infection by wild-type VSV on L-cell protein synthesis.

To learn more about the properties of protein synthesis inhibition by the R1 mutant, we compared the multiplicity dependence of this mutant with that of the wild-type virus. The data (Fig. 5) showed a large difference between the wild type and the mutant in the MOI needed to inhibit protein synthesis. Three experiments with the wild-type virus are presented to demonstrate that regardless of the extent of protein synthesis inhibition, 63% of the maximal inhibition was present at an MOI of 1 PFU per cell. This is compatible with a one-hit kinetic model with respect to PFU per cell. The maximal inhibition of protein synthesis for R1 was not reached until the MOI was 500 PFU per cell (data not shown). These results fit a kinetic model in which approximately 60 PFU per cell are necessary to obtain 63% of the maximum inhibition. At the

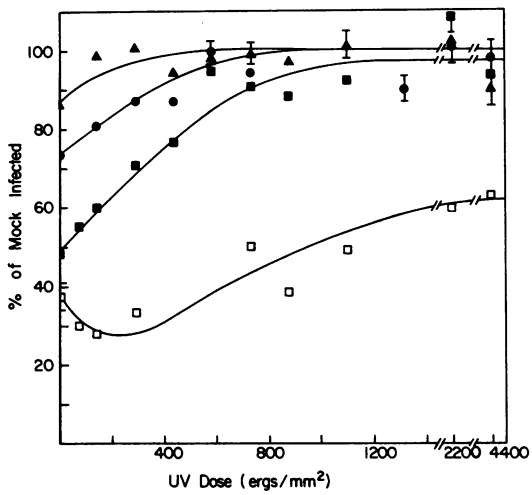

FIG. 4. Effect of UV-irradiated wild-type and R1 virus on L-cell protein synthesis. L-cells were treated as described in the legend to Fig. 1. Virus was irradiated to the indicated degree. The infected or mock-infected cells were incubated for 3.5 h at 37°C and then pulse-labeled with L-[^{35}S]methionine in complete medium plus bovine calf serum for 0.5 h at 37°C. The infection was quenched, and the medium was extracted with 0.1 N NaOH, as described in the text. The upper three curves represent infections with the R1 virus at three MOIs: (▲) 2.4; (●) 43; (■) 300. The lower curve represents an infection with the wild-type virus at an MOI of 13.

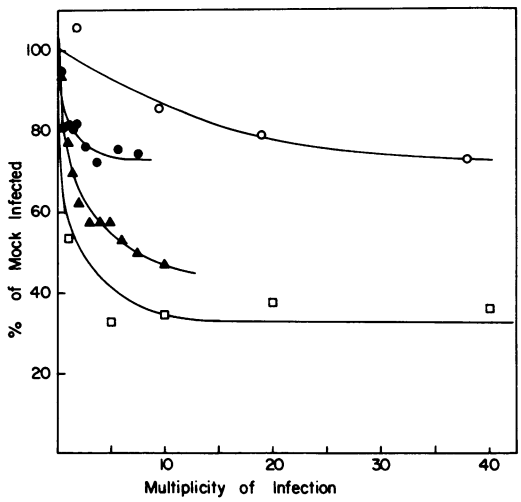

FIG. 5. Effect of MOI on protein synthesis inhibition in L-cells infected by wild-type VSV or by the R1 mutant. L-cells were treated as described in the text. Virus was diluted and used to infect L-cells for both protein synthesis and infectivity measurements. The abscissa was corrected to the measured titer for each experiment. The lower three curves represent three experiments with wild-type virus done on different dates. The upper curve represents an experiment with the R1 virus.

multiplicities of R1 tested, there was no measurable inhibition that could be attributed to the small transcription product (see Fig. 4).

Target sizes. The target sizes of the UV-sensitive regions of the wild-type and of the R1 mutant genomes were determined as described above. The upper two curves in Fig. 6 represent the inactivation of the large transcription product inhibitor of protein synthesis. The size of the wild-type virus inhibitor was calculated to be 373 ± 71 nucleotides and that of the R1 mutant inhibitor was calculated to be 807 ± 125 nucleotides. The solid line in Fig. 6 represents the loss of infectivity as a function of UV dose.

Figure 7 shows the data for the inactivation of the small transcription product of the wild-type virus. The statistical analysis was as described for Fig. 6. The target size was 42 ± 12 nucleotides.

DISCUSSION

This report shows our attempts to define more precisely the region(s) of the VSV genome necessary for protein synthesis inhibition, using the technique of UV mapping. Wertz and Youngner (29) suggested that two mechanisms are involved in the inhibition of cell protein synthesis by VSV: an initial, UV-insensitive inhibition and a progressive, UV-sensitive inhibition. No UV-insensitive inhibition of protein synthesis was detected in our studies. In fact, the UV inactivation curve for protein synthesis inhibition shown in this paper was biphasic, suggesting that two transcription products are involved in the inhibition. The target sizes of the two UV-sensitive regions of the VSV genome were calculated to be approximately 375 and 42 nucleotides. The large transcription product inhibitor accounted for 35 to 40% of the total inhibition, and the small transcription product inhibitor accounted for 60 to 65% of the total inhibition. Neither of these putative inhibitors corresponded in size to any of the known viral mRNA species.

The origin of these transcripts is uncertain. Recent reports have noted the presence of several small RNA species during in vitro transcription (9, 20, 21, 23, 26). These small RNAs arise from the 3'-proximal region of several of the viral genes and represent the initiation of transcription at defined sites. Their initiation is independent of that of the leader RNA (5). Some of the small RNA transcripts are capped (9, 23, 26) and are highly resistant to UV irradiation (9, 26; J. D. Keene, personal communication). The capped, UV-resistant RNA species are tran-

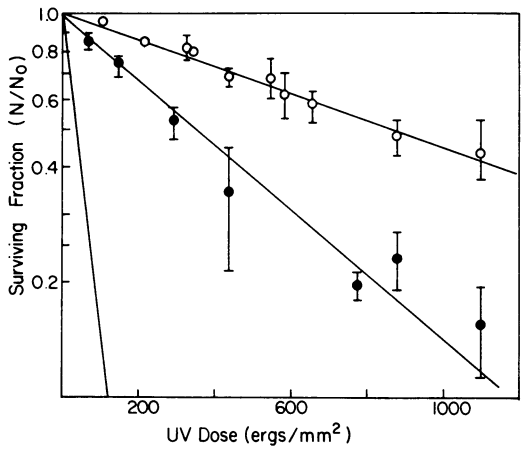

FIG. 6. Target size analysis of the large transcription product of the VSV genome involved in protein synthesis inhibition. Target size analysis is described in the text. The loss of infectivity as a function of UV dose is indicated by the steep solid line. The slope of this curve was -9.81×10^{-3} mm²/erg for the wild-type virus and -8.42×10^{-3} mm²/erg for the R1 mutant. The loss of protein synthesis inhibition as a function of UV dose is indicated by the data for the wild-type virus (○) and the R1 mutant (●). The slope of the curve was $-3.56 \times 10^{-4} \pm 0.70 \times 10^{-4}$ mm²/erg for the wild-type virus and $-7.70 \times 10^{-4} \pm 1.25 \times 10^{-4}$ mm²/erg for the R1 mutant. These data represent a composite of six and two independent experiments, respectively. The data points are the means of the data collected, and the error bars are the standard deviations of the data collected. The curve drawn through the points is a least-squares best-fit analysis of all of the data collected.

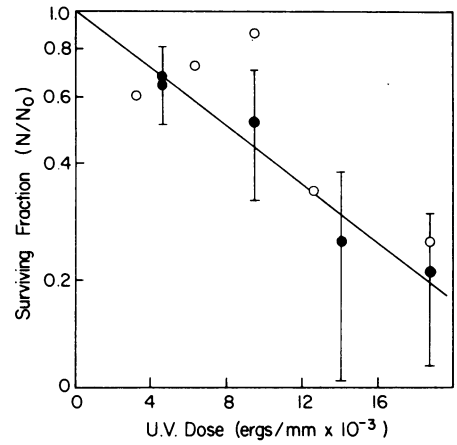

FIG. 7. Target size analysis of the small transcription product of the VSV genome involved in protein synthesis inhibition. The target size analysis is described in the text. The loss of protein synthesis inhibition as a function of UV dose is depicted by two independent experiments: (○) an experiment in which only one data point was taken at each irradiation dose; (●) a second experiment in which four data points were taken at each irradiation dose and averaged. The slope of this curve is $-4.5 \times 10^{-5} \pm 1.08 \times 10^{-5}$ mm²/erg. The statistical analysis was as described in the legend to Fig. 6.

scribed from the 3'-proximal region of the N protein gene and contain a potential ribosome binding site (7, 22). Because of their similarity in size and UV resistance, these small RNA species may correspond to the putative small transcription product inhibitor reported in this paper. If so, the effect of the inhibitor on protein synthesis could be to tie up ribosomes, since initiation of protein synthesis could conceivably occur on the fragment. Since there are no chain termination signals on the fragment, ribosomes would remain stalled, and the 40S and 60S subunit pools would be depleted.

The putative large transcription product (375 nucleotides) could also be derived from the N protein gene in view of the recent isolation and characterization by Banerjee and co-workers (25; personal communication) of a 9S viral RNA species corresponding to the 5'-terminal transcription product of the N protein gene. This 9S RNA species is present in vivo and in vitro, contains approximately 400 nucleotides, is capped, has three potential ribosome binding sites, and is transcribed in equal molar quantities relative to the N protein mRNA.

VSV infection results in the inhibition of cellular RNA synthesis in addition to cellular protein synthesis (28, 30). The current evidence suggests that the leader RNA, which contains 48 nucleotides (5), is involved in this process (11, 19). If efficient protein synthesis is dependent on continuous RNA synthesis, then any perturbation in the rate of RNA synthesis would affect the rate of protein synthesis. Thus, should the putative small transcription product be identified as leader RNA, its effect on protein synthesis could be a consequence of RNA synthesis inhibition.

These possible mechanisms are only speculative, and there is no evidence to favor one over another. It is probable that more than one mechanism is operating, in view of our results with the R1 mutant. We are in the process of identifying the putative transcription products described in this report.

The R1 mutant described by Stanners et al. (24) poses an interesting problem. As shown in Fig. 4, 5, and 6, the R1 mutant behaved quite differently from the wild-type virus in a number of respects. First, a much higher MOI was needed to elicit inhibition of protein synthesis (see Fig. 5). One infectious particle per cell was sufficient for 63% inhibition by the wild-type virus, whereas as many as 60 infectious particles per cell were needed by the mutant. Secondly, the UV inactivation curve was monophasic in the case of the mutant (Fig. 4), as opposed to being biphasic in the case of the wild-type virus. It is possible that the P function is related to the role of the small transcription product. Our preliminary experiments show that in cells infected with the R1 mutant, RNA synthesis inhibition occurred to a much lesser extent than in cells infected with the wild-type virus. These findings suggest that there is some link between RNA and protein synthesis inhibition.

These studies have also reinforced our contention (10) that the inhibition of protein synthesis by VSV does not result from competition between viral and cellular mRNA species for available ribosomes in the cell (13, 14). Until a very high dose of UV irradiation was applied to VSV, the virus was still capable of inhibiting protein synthesis. Under these conditions little, if any, viral mRNA synthesis occurred, as ascertained by the low amount of viral proteins synthesized (see Fig. 1B and Fig. 3).

The comparative study (Fig. 3 and Table 1) of the translation of the protein products synthesized by unirradiated and UV-irradiated VSV in vivo and in vitro showed that the same species of proteins were made. This finding will allow us to study this inhibition in vitro.

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