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# Use of UV Irradiation to Identify the Genetic Information of Vesicular Stomatitis Virus Responsible for Shutting Off Cellular RNA Synthesis

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UV irradiation of infectious vesicular stomatitis virus was employed to study the relationship between the expression of certain viral gene functions and viral inhibition of RNA synthesis in mouse myeloma (MPC-11) cells. Viral infectivity, protein synthesis, and viral mRNA synthesis were all highly susceptible to inactivation by UV radiation; however, low levels of viral transcriptase activity were detected in vitro in virus preparations subjected to large doses of UV radiation. In sharp contrast, the capacity of vesicular stomatitis virus to shut off cellular transcription was quite resistant to UV radiation. The data presented here indicate that viral transcription is essential to inhibit host RNA metabolism, even though synthesis of viral polypeptides in the inhibited cells could not be detected. At those levels of UV radiation that inactivated all viral gene functions, except viral inhibition of cellular RNA synthesis, the only viral product detected was non-adenylated, low-molecular-weight RNA species.

Inhibition of cellular macromolecular synthesis by vesicular stomatitis (VS) virus evidently requires the synthesis of viral transcriptional, and possibly translational, products (10, 13, 19). Previous work in this laboratory has shown that viral inhibition of cellular RNA metabolism takes place at the level of nuclear polymerases (16) and is dependent on primary transcription of the viral genome (17); input virion components alone do not appear to be capable of shutting off cellular transcriptional processes. This observation also appears to hold true for the inhibition of protein synthesis in the host cell (10, 13). Recent work by Marvaldi et al. (12), utilizing UV-irradiated VS virus, implied that minimal amounts of viral N and NS proteins must be produced for the virus to shut off protein synthesis of the host and kill cells. In contrast, VS viral inhibition of cellular nucleic acid metabolism appears to be quite resistant to high doses of UV radiation, even though viral infectivity as measured by plaque assays is extremely susceptible to inactivation by UV light (9).

The studies reported here were performed to examine more closely the involvement of viral RNA and protein synthesis in the inhibition of host RNA metabolism. Since earlier experiments with temperature-sensitive (*ts*) mutants and defective-interfering particles of VS virus demonstrated that viral transcription was a prerequisite to cellular shutoff (10, 13, 17), we sought to determine which viral gene(s) need to be transcribed to compromise cellular functions. Since the VS virus genome is apparently transcribed sequentially from the 3′ end (1, 2), UV irradiation of virus stocks allows a preferential destruction of viral gene functions. The present data reveal that viral mRNA and protein synthesis are lost at very low levels of UV irradiation, whereas the inhibitory effect of the virus is relatively resistant to inactivation. These observations are discussed in terms of which viral gene function is required for inhibition of host RNA synthesis.

## MATERIALS AND METHODS

**Chemicals and radioisotopes.** [$^3$H]uridine (26 Ci/mmol) and [$^{14}$C]rRNA markers were purchased from New England Nuclear Corp., Boston, Mass. [$^3$H]UTP (12 Ci/mmol) was purchased from Schwarz/Mann, Orangeburg, N. Y. [$^{14}$C]protein lysate (56 mCi/matom) and Nuclear-Chicago solubilizer were obtained from Amersham/Searle, Arlington Heights, Ill. Nucleoside triphosphates, Triton X-100, and dithiothreitol were obtained from Sigma Chemical Co., St. Louis, Mo. Electrophoresis-grade agarose, acrylamide, *N,N′*-methylenebisacrylamide, and sodium dodecyl sulfate were provided by Bio-Rad Laboratories, Richmond, Calif. Nonidet P-40 is a product of Shell Oil Co. *N,N,N′,N′*-tetramethylethylenediamine was purchased from Eastman Kodak, Rochester, N. Y. The oligodeoxythymidylic acid [oligo(dT)]-cellulose was obtained from Collaborative Research, Inc., Waltham, Mass.

**Cells, virus, and media.** Mouse myeloma cells, MPC-11, were cultured as described previously (16).

All tissue culture supplies were obtained from Grand Island Biological Co., Grand Island, N. Y.

The VS virus, strain San Juan (Indiana serotype), used in these experiments was grown on BHK-21 cells from clones of virus selected from plaques picked from L-cell monolayers. This virus strain was originally obtained from the U.S. Agricultural Research Center, Beltsville, Md. (15). The resulting virus stocks were titrated by assay of PFU on monolayers of L cells and were stored at −80°C.

**UV irradiation of virus.** Stock preparations of VS virus ($10^9$ PFU/ml) were diluted 1:10 in 1 ml of Dulbecco-modified phosphate-buffered saline and dispensed into a standard tissue culture dish (60 by 15 mm). Dishes were placed at a distance of 10 cm from the UV light source and exposed to radiation at a wavelength of 254 nm and at a dose rate of 85 ergs/$mm^2$ per s. As measured by PFU, the 37% (1/e) survival dose for VS virus infectivity was 104 ergs/$mm^2$. After irradiation, the virus preparations were used to infect myeloma cells at a multiplicity of infection of 10, based on the original PFU titer of the virus stocks before irradiation. The virus was adsorbed to cells for 30 min at room temperature, and the cells were pelleted by centrifugation and resuspended in complete media and maintained at 37°C in 10-ml Spinner cultures.

The irradiation of purified VS virus before testing in vitro transcriptase activity was performed under similar conditions. After purification and pelleting of VS virus as described previously (8), the viral pellet was resuspended at a protein concentration of 1 mg/ml in reticulocyte standard buffer containing 15% glycerol. A 1-ml amount of this virus suspension was dispensed into tissue culture plates (60 × 15 mm) and subjected to UV radiation as described above. After this treatment, the virus preparations were tested at a protein concentration of 0.3 mg/ml under standard transcriptase assay conditions (5).

**Measurement of cellular RNA synthesis.** Decline of cellular RNA metabolism after infection with irradiated or unirradiated VS virus was monitored as described previously (16). Briefly, samples of cells were removed from Spinner cultures at hourly intervals postinfection and pulse-labeled in media containing 2 μCi of [$^3$H]uridine per ml. RNA synthesis was measured as the amount of radioactivity incorporated into cold trichloroacetic acid-precipitable material.

**Assays of viral RNA synthesis.** The in vitro assays for viral transcriptase activity were performed as detailed by Carroll and Wagner (5). Reactions were incubated at 31°C in stoppered tubes, and the reaction mixtures contained the maximal concentrations of virus which did not result in endogenous inhibition of the transcriptase (6). After a 60-min incubation period, ice-cold 25% trichloroacetic acid was added, and the acid-insoluble RNA was measured by scintillation spectrometry.

The measurement of VS viral RNA synthesis in mouse myeloma cells was performed by the procedure of Wertz (18). At 1.5 h postinfection, cells were incubated for 30 min in media containing 20 mM glucosamine and 5 μg of actinomycin D per ml. After this pretreatment, the cells were pelleted by centrifugation and resuspended in warm media containing 2 μCi of [$^3$H]uridine and 5 μg of actinomycin D per ml and reincubated for 20 min. Immediately after the labeling period, the cells were fractionated by Nonidet P-40 lysis as described previously (16). The resulting supernatant fluid was carefully decanted and extracted with cold phenol at pH 9 as described by Brawerman (4), and RNA was precipitated in ethanol at −20°C.

The [$^3$H]RNA was fractionated by affinity chromatography on oligo(dT)-cellulose in a 1-ml syringe column equilibrated with buffered 0.5 M NaCl. The RNA was solubilized in 0.5 ml of buffer containing 0.01 M Tris-hydrochloride (pH 7.4), 0.5 M NaCl, and 0.05% sodium dodecyl sulfate and applied to the column, and the non-adenylated RNA was washed through. The polyadenylated [poly(A)] RNA was eluted from the column in a salt-free buffer and reprecipitated by the addition of two volumes of ethanol. The resulting RNA precipitate was analyzed by polyacrylamide gel electrophoresis.

**Analysis of RNA products.** Viral poly(A) RNA products were separated by electrophoresis in cylindrical gels containing 2% acrylamide and 1% agarose as described previously (16). The gels were sectioned into 1-mm slices on a Bio-Rad model 195 electric gel slicer and solubilized in 0.5 ml of Nuclear-Chicago solubilizer, and radioactivity was measured in toluene-based scintillation fluid.

**Analysis of protein synthesis.** Proteins synthesized by infected and mock-infected cells were analyzed by slab gel electrophoresis (5). At 3.5 h postinfection, $10^6$ infected or uninfected cells were incubated in 1 ml of a buffer containing Dulbecco-modified Eagle medium diluted 50:1 in phosphate-buffered saline and 2 μCi of $^{14}$C-protein hydrolysate per ml. After incubation for 30 min at 37°C, the cells were pelleted by centrifugation, washed twice in ice-cold phosphate-buffered saline and solubilized in 100 μl of sample buffer containing 1.4% dithiothreitol, 4% sodium dodecyl sulfate, 6.25 mM Tris-hydrochloride (pH 6.8), and 0.01% bromophenol blue. After heating at 100°C for 3 min, the samples were loaded onto discontinuous Tris–glycine-buffered sodium dodecyl sulfate gels consisting of a 12.5% acrylamide resolving gel and a 5% acrylamide stacking gel. After electrophoresis, the slab gels were dried, and autoradiograms were prepared by exposure on Kodak SB-54 medical X-ray film. The resulting autoradiograms were scanned on a Schoefel model SD 3000 Spectrodensitometer. The relative concentrations of the different viral proteins were determined by measuring the areas under the peaks on the scan and were compared with the amount of protein synthesized by unirradiated VS virus.

## RESULTS

**UV inactivation of VS viral inhibitory activity.** Figure 1 illustrates the dose effect of UV irradiation on the ability of VS virus to shut off cellular RNA synthesis at 4 h after infection of myeloma cells. Up to a UV dose of 15,000 ergs/$mm^2$, the virus stocks retained approximately 95% of maximal inhibitory activity, and no significant loss of this activity was detected until about 30,000 ergs/$mm^2$ was administered to the virus before infection. It appears, there-

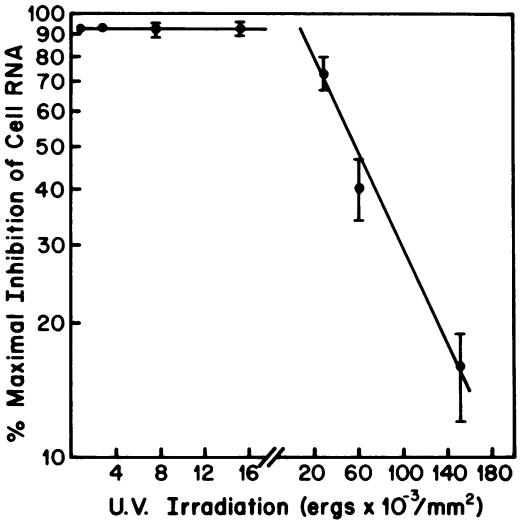


FIG. 1. *Effect of UV irradiation on VS virus inhibition of mouse myeloma cellular RNA synthesis. After irradiation of VS virus as described in the text, MPC-11 cells were infected at a multiplicity of ≃10 PFU/cell and maintained in 10-ml Spinner cultures at 37°C. At 4 h postinfection, duplicate portions of 5 × 10⁵ infected or mock-infected cells were removed and incubated for 15 min at 37°C in 1 ml of medium containing 2 μCi of [³H]uridine per ml. RNA synthesis was measured as the amount of radioactivity incorporated into trichloroacetic acid-insoluble material and calculated as 6.25 pmol of uridine incorporated per mg of protein. The percent inhibition produced by unirradiated VS virus was 90% and was designated as maximal inhibition. The data are presented as a percentage of maximal inhibition, and the vertical bars represent the range in triplicate values for that point.*

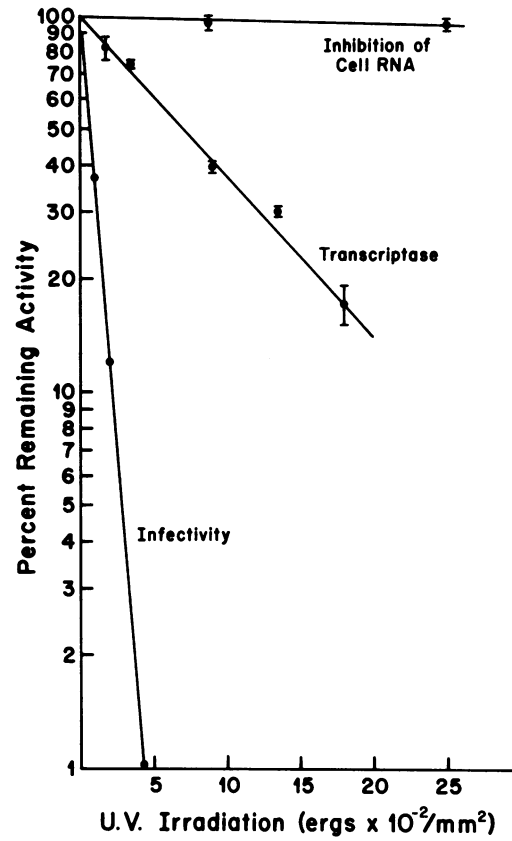


FIG. 2. *Comparative effects of UV irradiation on VS viral infectivity and in vitro transcriptase activity as well as inhibition of cellular RNA synthesis. Stock preparations of VS virus containing 10⁸ PFU/ml were irradiated and subsequently used to infect monolayers of L cells and scored for residual PFU (infectivity). In separate experiments, purified VS virus was subjected to increasing doses of UV irradiation before measuring viral transcriptase activity in vitro. Unirradiated virus incorporated 8.8 nmol of [³H]UMP per mg of protein per h of incubation at 31°C. The data are expressed as the percentage of PFU or transcriptase activity detected in virus preparations receiving no UV radiation. For reference, a portion of the data presented in Fig. 1 is replotted at the top of the figure (inhibition). When applicable, the range of values for a particular point is designated by vertical bars.*

fore, that the capacity of VS virus to shut off cell RNA synthesis is extremely resistant to UV inactivation; even doses of UV greater than 150,000 ergs/mm² did not completely abolish the capacity of VS virus to inhibit cellular RNA synthesis.

**Effect of UV irradiation on VS viral infectivity and transcriptase activity.** The relationship between UV dose and loss of certain viral functions was examined by measuring the infectivity of stock virus preparations and the in vitro transcriptase activity of purified virus after exposure to UV radiation. As reported previously (9), the infectivity of VS virus stock preparations was extremely sensitive to UV irradiation. Figure 2 shows that the 37% (1/e) survival dose for plaque-forming particles was 104 ergs/mm². A UV dose of 850 ergs/mm² resulted in complete elimination of the capacity of $10^8$ PFU of VS virus to form plaques on monolayers of L cells (data not shown). Thus, at those levels of UV irradiation that destroyed viral infectivity, there was essentially no effect on the ability of VS virus to inhibit cellular RNA synthesis (compare Fig. 1 and 2).

Since these low levels of UV irradiation completely inactivate the infectivity of VS virus, it was of interest to compare the direct influence of UV irradiation on in vitro transcription. Even when infectivity was lost, it seemed likely that

viral transcription could still occur. In addition, it has been shown that the synthesis of the VS virus leader RNA sequence from the 3' end of the genome is highly resistant to UV irradiation (7). Figure 2 shows that in vitro viral transcriptase activity of VS virus is not as sensitive to UV inactivation as is the overall infectivity. The 37% survival dose for transcriptase activity was found to be 1,050 ergs/mm$^2$, which agrees closely with the data of Ball and White (2). However, even at doses of 51,000 ergs/mm$^2$, purified VS virus retained approximately 0.1% of the transcriptase activity of unirradiated VS virus (data not shown). These results indicate that at those UV doses (≥55,000 ergs/mm$^2$) which reduce the inhibitory effect on cell RNA synthesis by 50% (Fig. 1), there are very low levels of residual transcriptase activity.

**Effect of UV irradiation on viral mRNA and protein synthesis in infected MPC-11 cells.** Although the above experiments demonstrated that in vitro viral transcription could be progressively reduced by UV irradiation, it was necessary to determine whether UV irradiation also inactivated synthesis of viral mRNA in infected cells. To examine effects on in vivo viral RNA synthesis, VS virus stocks were irradiated before infecting cells; at 2 h postinfection, cells were pulse-labeled for 15 min in media containing [$^3$H]uridine and actinomycin D. The cytoplasmic RNA from cells infected with unirradiated or UV-irradiated VS virus was phenol extracted, ethanol precipitated, and separated on oligo(dT)-cellulose columns.

Figure 3 compares the profiles by polyacrylamide gel electrophoresis of the poly(A) viral RNAs present in cells infected with unirradiated and UV-irradiated VS virus. As noted, unirradiated virus gave rise to [$^3$H]RNA separated into three distinct peaks. The major peak of viral RNA migrated at ~18S and represents the viral messengers coding for the N and G proteins. The faster migrating peak at about 13S represents M and NS mRNA's, and the slowest migrating peak at ~31S codes for the viral L protein (transcriptase). Very low levels of UV (170 ergs/mm$^2$) irradiation of the virus before infection resulted in a marked reduction of all viral mRNA; a UV dose of 425 ergs/mm$^2$ resulted in almost total loss of detectable poly(A) mRNA species (Fig. 3). As observed for viral infectivity, mRNA synthesis by the virus was extremely sensitive to inactivation by UV irradiation (compare Fig. 2 and 3).

Although there were very low levels of poly(A) RNA synthesis after a UV dosage of 425 ergs (~1% of total [$^3$H]RNA synthesized in uninfected cells), there was still a measurable degree

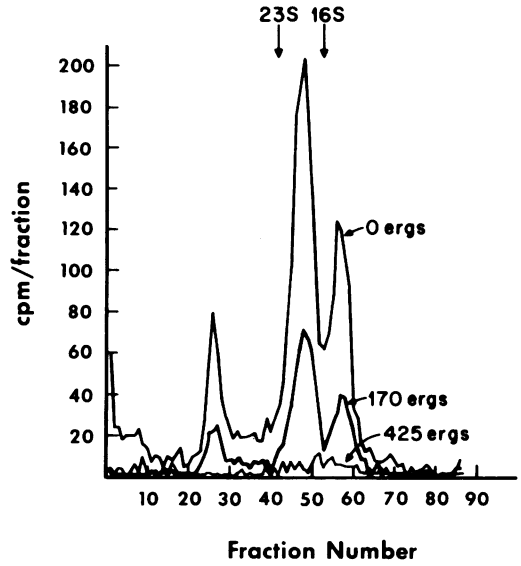


FIG. 3. *Electropherograms of poly(A) viral RNA synthesized in myeloma (MPC-11) cells infected with unirradiated (0 ergs) VS virus or with VS virus UV irradiated with 170 or 425 ergs/mm$^2$. After irradiation of virus with the doses indicated, myeloma cells were infected, and viral RNA was labeled for 20 min with [$^3$H]uridine at 2 h postinfection as described in the text. After this incubation, the cells were lysed with Nonidet P-40 detergent, and the postnuclear supernatant was extracted with phenol and the RNA was precipitated in ethanol. The resulting RNA was separated on an oligo(dT)-cellulose column, reprecipitated, and subjected to polyacrylamide gel electrophoresis. The RNA samples represented the total amount of poly(A) RNA which could be extracted from equal numbers of infected myeloma cells. The arrows represent internal [$^{14}$C]RNA markers derived from E. coli ribosomes.*

of [$^3$H]uridine incorporated into extractable nonadenylated RNA products. In the experiment depicted in Fig. 3, unirradiated VS virus gave rise to nonadenylated [$^3$H]RNA of 107,720 cpm compared with 74,360 cpm of [$^3$H]RNA from cells infected with viral preparations receiving 425 ergs of UV radiation. Sucrose gradient analysis of these non-adenylated RNA species revealed that they were all less than 4S in size (data not shown). Therefore, at levels of UV which destroyed most of the poly(A) viral mRNA synthesis, there was still considerable production of low-molecular-weight, non-adenylated RNA molecules.

**Effect of UV irradiation on VS viral protein synthesis.** Since viral mRNA synthesis is exquisitely sensitive to UV irradiation, viral protein synthesis should be comparably impaired by UV irradiation. To determine the degree to which viral polypeptide synthesis is altered by

UV irridation, myeloma cells were infected with unirradiated VS virus or virus that had been subjected to various doses of UV; at 3.5 h postinfection, cells were radioactively pulse-labeled for 15 min with $^{14}$C-amino acids. The resulting radioactive polypeptides were extracted and separated by slab gel electrophoresis.

Figure 4 shows a typical autoradiogram of VS viral proteins synthesized in cells infected with differentially irradiated VS virus. This procedure clearly demonstrates that detectable levels of viral proteins are lost at very low levels of UV irradiation. When the virus was exposed to 425 ergs/mm$^2$, there was a drastic reduction in the synthesis of all viral proteins, with only a minor portion of N protein remaining as compared with unirradiated virus (0 ergs/mm$^2$). However, even complete suppression of all viral protein synthesis at a dose of 850 ergs/mm$^2$ did not affect the ability of VS virus to inhibit cellular protein synthesis (compare in Fig. 4 the amount of cellular protein labeled in infected and mock-infected cells). This level of UV irradiation (850 ergs/mm$^2$) is markedly less than the minimal amount (30,000 ergs/mm$^2$) required to reduce significantly the viral inhibition of host RNA synthesis.

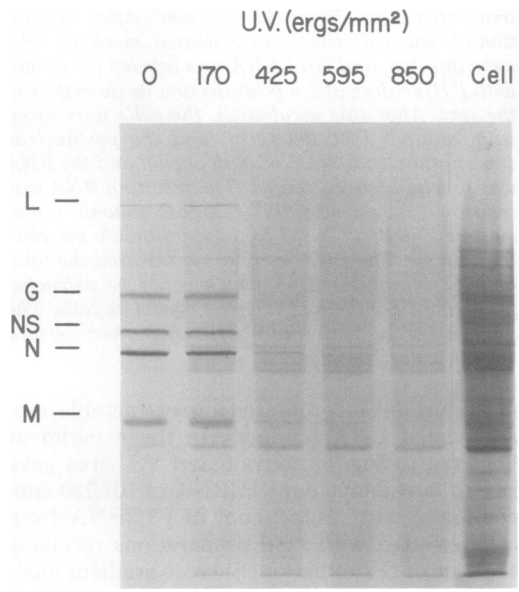


FIG. 4. *Autoradiograms of proteins synthesized in mouse myeloma (MPC-11) cells infected with unirradiated or UV-irradiated VS virus. Before infection of myeloma cells, VS virus was UV irradiated with the various doses indicated. At 3.5 h postinfection, cells were pelleted, resuspended in buffer containing 2 μCi of $^{14}$C-amino acid mixture per ml, and reincubated for 30 min at 37°C. After this incubation, the cell lysates were prepared and subjected to slab gel electrophoresis, and an autoradiogram of the resulting gel was developed as described in the text. The track labeled "cell" contained proteins synthesized by mock-infected myeloma cells, and the position of viral protein markers is depicted on the left.*

**Comparative susceptibility to UV irradiation of various VS viral functions.** Figure 5 summarizes in quantitative terms the amount of UV irradiation required to compromise VS viral mRNA and N protein synthesis compared with the amount required to inhibit cellular RNA synthesis. These data were obtained by integration of the viral poly(A) mRNA peaks shown in Fig. 3 and the densitometry scans of N protein as shown in Fig. 4, as well as the viral loss of cellular RNA inhibitory activity shown in Fig. 1.

Extrapolation of these data indicates that only 1% of maximal N protein synthesis would remain after exposure to 1,600 ergs/mm$^2$ of UV irradiation. Since the gene coding for the N protein is the viral genome region most resistant to UV inactivation (1, 2), this calculation represents a conservative estimate of the loss of total viral protein synthesis. In comparison, it would take an approximate 20-fold increase in UV dose to exert even a slight effect on viral inhibition of host RNA biosynthesis (Fig. 1). In a similar fashion, viral poly(A) RNA declined rapidly with increasing doses of UV at an initial rate even greater than that for protein synthesis (Fig. 5). As stated previously, however, there were still significant amounts of low-molecular-weight, non-adenylated RNA species detected in the cytoplasmic fraction of cells infected with UV-irradiated VS virus.

## DISCUSSION

The results presented in this paper demonstrate that the inhibition of mouse myeloma cellular RNA synthesis caused by VS virus is highly resistant to inactivation by UV radiation. In contrast, viral infectivity, transcription of the viral genome into messenger-length RNA, and subsequent production of viral polypeptides were found to be quite sensitive to UV radiation. Table 1 illustrates the striking comparison of the sensitivity to UV irradiation of the cell inhibitory factor and of other viral functions.

Previous observations by other investigators led to the conclusion that input virion components alone can inhibit cell macromolecular synthesis without the apparent synthesis of new viral products (3, 9, 20). Baxt and Bablanian (3) observed that UV-irradiated VS virus retained its ability to inhibit cellular protein synthesis, whereas heat-inactivated virus did not. A comparable finding was reported by Yaoi et al. (20)

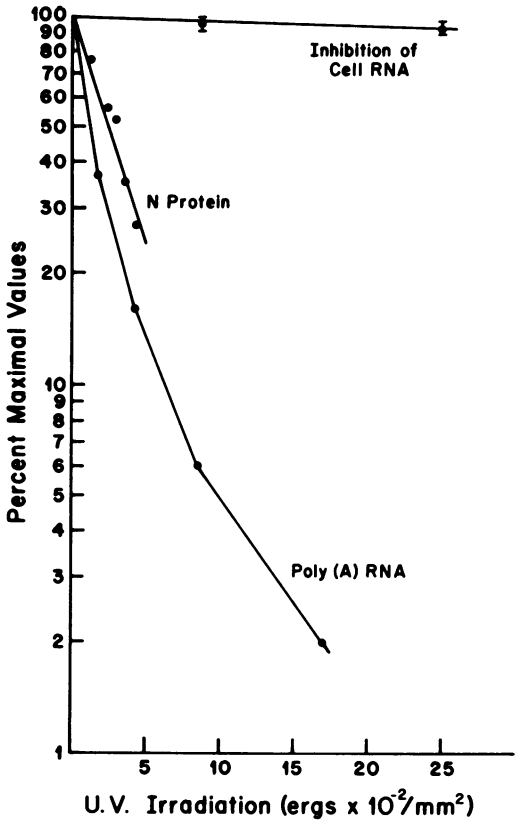


FIG. 5. *Comparative effects of UV irradiation on the capacity of VS virus to synthesize mRNA and N protein in MPC-11 cells and to inhibit cellular RNA synthesis. Stock preparations of VS virus were exposed to various doses of UV irradiation and used to infect equal numbers of mouse myeloma cells. Viral mRNA was measured by labeling $10^7$ cells with [$^3$H]uridine (2 μCi/ml) at 37°C for 20 min at 2 h postinfection in the presence of actinomycin D (5 μg/ml). Viral protein synthesis was measured in separate experiments by labeling $10^6$ cells with $^{14}$C-protein hydrolysate (2 μCi/ml) for 30 min at 3.5 h postinfection. The resulting radioactive RNA or protein was isolated as described in the text. The labeled polypeptides were subjected to polyacrylamide gel electrophoresis, and an autoradiogram was prepared and scanned. The [$^3$H]RNA was subjected to oligo(dT)-cellulose column chromatography, and the amount of poly(A) RNA was determined. Maximum amount of poly(A) RNA was 5,750 cpm. The inhibition of cellular RNA synthesis by virus receiving two different doses of radiation is plotted at the top of the figure (inhibition). All data are expressed as the percentage of the inhibitory activity or viral product of unirradiated VS virus.*

for the effect of VS virus on cellular nucleic acid synthesis. These investigators (3, 20) provided no information on wavelength or dose of UV irradiation. Huang and Wagner (9), with the same UV light source reported here, showed that the inhibitory activity of VS virus on Krebs-2 ascites cell RNA synthesis was resistant to UV radiation up to a dose of 60,000 ergs/mm$^2$; UV irradiation of ~150,000 ergs/mm$^2$ reduced cell RNA-inhibiting activity by 70%. Although UV radiation drastically alters the ability of VS virus to replicate, recent work on mapping the VS virus genome has revealed that transcription of a very short sequence from the 3′ end of the genome RNA is highly resistant to inactivation by UV irradiation (7). Therefore, it seems possible that earlier studies involved the use of UV-irradiated VS virus that could still transcribe a very limited portion of the viral genome. In the present study, the examination of in vitro transcriptase activity, as well as viral RNA synthesis in myeloma cells, revealed that residual in vitro RNA synthesis could be detected after exposing VS virus to high levels of UV radiation and that the in vivo transcription products were low-molecular-weight, non-adenylated RNA molecules. Thus, we propose that transcription of a small segment of the viral genome might be required for inhibition of cellular RNA metabolism. However, once the activity of the viral transcriptase enzyme is destroyed (e.g., by heat inactivation), the inhibitory activity of the virus would also be lost, as observed by Baxt and Bablanian (3) and Yaoi et al. (20). It is interesting to note that very large doses of UV radiation are required to destroy viral inhibition of host RNA synthesis (Fig. 1). Such levels of UV radiation may be high enough to act directly on protein molecules and alter the enzymatic activity of the viral transcriptase. In any event, the amounts of UV irradiation needed to reduce the inhibitory capacity of VS virus are far greater than those needed to inactivate the mRNA synthesis on the viral genome.

Marcus et al. (10) have suggested a similar

TABLE 1. *Comparative effect of UV irradiation on the survival of different VS viral functions*

| VS viral function | UV dose resulting in 63% loss in activity (ergs/mm$^2$)[a] |
|---|---|
| Infectivity (PFU/ml) | 104 |
| Transcriptase (in vitro) | 1,050 |
| Viral mRNA (in vivo) | 170 |
| N protein (in vivo) | 380 |
| Shutoff of host RNA | 72,000 |

[a] Based on the data presented in Fig. 1 to 5, the 37% (1/e) survival rates of the various viral functions were determined. The data are expressed as the dosage of UV irradiation at which 37% of the maximal viral activity or viral product could still be detected as described in the individual experiments.

mechanism for VS virus inhibition of cellular protein synthesis, by which a portion of the genome immediately adjacent to the leader sequence must be transcribed. Previous work in our laboratory has shown that defective-interfering particles fail to shut off cellular RNA metabolism (17), a finding similar to that for cell killing (11). Such defective-interfering particles are capable of transcribing in vitro only a leader sequence 46 nucleotides in length (14); this defective-interfering leader sequence is quite distinct from the leader sequence read off the 3′ end of the complete VS-B virion genome (7). In addition, *ts* mutants of VS virus blocked in primary transcription do not inhibit host RNA metabolism at the temperature nonpermissive for viral RNA synthesis (17). These results indicate that viral transcription is a prerequisite for shutting off cellular RNA and possibly protein synthesis, but that the defective-interfering leader sequence alone is not sufficient to induce the inhibition. Whether the transcriptional products must be longer than this particular segment or must differ in nucleotide sequence is presently not known. Conceivably, the VS viral inhibitor of cellular macromolecular synthesis could be the leader RNA transcribed off the 3′ end of the complete viral genome.

The inhibition of cellular RNA metabolism apparently does not require the synthesis of new viral proteins. In contrast to the inhibition of cellular protein synthesis (12), the sensitivity to UV irradiation of viral N protein synthesis does not correlate well with the loss of cell RNA inhibitory activity after irradiation of VS virus (Fig. 5). Detectable levels of N protein synthesis are lost at doses of UV radiation that are at least 21-fold less than those required to cause only the slightest reduction in the cell RNA inhibitory capacity of the virus. Such differences may be due to the fact that other investigators have examined cell killing and inhibition of host protein synthesis (10–12), compared with the present data which deal with inhibition of cellular RNA metabolism. In addition, differences in multiplicities of infection and sources of UV radiation may result in significant variations in virus inactivation. In the present studies VS virus was exposed to UV irradiation at a wavelength of 254 nm compared with the studies of Marvaldi et al. (12) performed at 275 nm, a wavelength which is more likely to have an effect on proteins such as the viral transcriptase. The site of action of the viral products may also be significant since inhibition of cellular RNA occurs at the level of the nuclear polymerases (16).

The shutoff of host RNA metabolism certainly requires a limited amount of transcription by infecting VS virus particles. Although the new product evidently exerts its effect within the nucleus of the cell (16), the exact nature of the cell-inhibiting viral transcript remains to be determined. Present work in our laboratory is being directed at more fully characterizing as candidates for the putative inhibitor the nonadenylated viral RNAs detected in cells infected with heavily UV-irradiated VS virus.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

1. **Abraham, G., and A. K. Banerjee.** 1976. Sequential transcription of the genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **73**:1504–1508.
2. **Ball, L. A., and C. N. White.** 1976. Order of transcription of genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **73**:442–446.
3. **Baxt, B., and R. Bablanian.** 1976. Mechanisms of vesicular stomatitis virus-induced cytopathic effects. II. Inhibition of macromolecular synthesis induced by infectious and defective-interfering particles. Virology **72**: 383–392.
4. **Brawerman, G.** 1974. The isolation of messenger RNA from mammalian cells. Methods Enzymol. **30**:605–612.
5. **Carroll, A. R., and R. R. Wagner.** 1978. Inhibition of transcription by immunoglobulin directed against the ribonucleoprotein of homotypic and heterotypic vesicular stomatitis viruses. J. Virol. **25**:675–684.
6. **Carroll, A. R., and R. R. Wagner.** 1978. Reversal by certain polyanions of an endogenous inhibitor of the vesicular stomatitis virus-associated transcriptase. J. Biol. Chem. **253**:3361–3363.
7. **Colonno, R. J., and A. K. Banerjee.** 1977. Mapping and initiation studies on the leader RNA of vesicular stomatitis virus. Virology **77**:260–268.
8. **Dubovi, E. J., and R. R. Wagner.** 1977. Spatial relationships of the proteins of vesicular stomatitis virus: induction of reversible oligomers by cleavable protein cross-linkers and oxidation. J. Virol. **22**:500–509.
9. **Huang, A. S., and R. R. Wagner.** 1965. Inhibition of cellular RNA synthesis by nonreplicating vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **54**:1579–1584.
10. **Marcus, P. I., and M. J. Sekellick.** 1975. Cell killing by viruses. II. Cell killing by vesicular stomatitis virus: a requirement for virion-derived transcription. Virology **63**:176–190.
11. **Marcus, P. I., M. J. Sekellick, L. D. Johnson, and R. A. Lazzarini.** 1977. Cell killing by viruses. V. Transcribing defective interfering particles of vesicular stomatitis virus function as cell-killing particles. Virology **82**:242–246.
12. **Marvaldi, J., M. J. Sekellick, P. I. Marcus, and J. Lucas-Lenard.** 1978. Inhibition of mouse L cell protein synthesis by ultraviolet-irradiated vesicular stomatitis virus requires viral transcription. Virology **84**:127–133.
13. **McAllister, P. E., and R. R. Wagner.** 1976. Differential inhibition of host protein synthesis in L cells infected with RNA$^-$ temperature-sensitive mutants of vesicular stomatitis virus. J. Virol. **18**:550–558.
14. **Schubert, M., J. D. Keene, R. A. Lazzarini, and S. U. Emerson.** 1978. The complete sequence of a unique

RNA species synthesized by a DI particle of VSV. Cell **15**:103–112.

15. **Wagner, R. R., A. H. Levy, R. M. Snyder, G. A. Ratcliff, Jr., and D. F. Hyatt.** 1963. Biological properties of two plaque variants of vesicular stomatitis virus (Indiana serotype). J. Immunol. **91**:112–122.
16. **Weck, P. K., and R. R. Wagner.** 1978. Inhibition of RNA synthesis in mouse myeloma cells infected with vesicular stomatitis virus. J. Virol. **25**:770–780.
17. **Weck, P. K., and R. R. Wagner.** 1979. Transcription of vesicular stomatitis virus is required to shut off cellular RNA synthesis. J. Virol. **30**:410–413.
18. **Wertz, G.** 1975. Method of examining viral RNA metabolism in cells in culture: metabolism of vesicular stomatitis virus. J. Virol. **16**:1340–1344.
19. **Wertz, G. W., and J. S. Youngner.** 1972. Inhibition of protein synthesis in L cells infected with vesicular stomatitis virus. J. Virol. **9**:85–89.
20. **Yaoi, Y., H. Mitsui, and M. Amano.** 1970. Effect of U.V.-irradiated vesicular stomatitis virus on nucleic acid synthesis in chick embryo cells. J. Gen. Virol. **8**: 165–172.