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Translation of Individual Host mRNA's in MPC-11 Cells Is Differentially Suppressed After Infection by Vesicular Stomatitis Virus

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Infection of MPC-11 mouse plasmacytoma cells by vesicular stomatitis virus results in a 30 to 35% reduction in [^{35}S]methionine incorporation into total proteins within 30 min postinfection. By 6 h postinfection, total protein synthesis is reduced by 80 to 90%. However, even by 30 min postinfection, a differential suppression of the synthesis of individual host protein is observed. The synthesis of the immunoglobin G (IgG) heavy chain (H), and, in particular, the synthesis of IgG light chain (L), is considerably more resistant to vesicular stomatitis virus-induced inhibition than is the synthesis of the non-IgG proteins as a whole; e.g., when the synthesis of non-IgG proteins was reduced by 41%, the synthesis of the H and L chains was reduced by 28 and 7%, respectively. Furthermore, these alterations in the relative synthesis of the L chain, H chain, and non-IgG are comparable to the alterations previously observed in uninfected MPC-11 cells when the overall rate of polypeptide chain initiation was selectively reduced (D. L. Nuss and G. Koch, 1976). These results are discussed in terms of the strategy of virus-directed suppression of host mRNA translation.

It has been known for some time that the synthesis of host proteins is decreased after infection by vesicular stomatitis virus (VSV) (19, 20). At low multiplicities of infection, viral replication appears to be required for inhibition of host protein synthesis to occur (21). However, at high multiplicities of infection, suppression of host mRNA translation proceeds in the absence of viral-specific transcription or translation, implicating the involvement of a preformed viral component in the shut-off mechanism (1, 22). Furthermore, studies measuring the effect of UV-irradiated VSV on L cell macromolecular synthesis have revealed a biphasic inhibition of total protein synthesis, i.e., an early UV-insensitive inhibition resulting in a 30% reduction in protein synthesis and a later UV-sensitive inhibition that appears to require the synthesis of viral mRNA (21). However, in the course of a productive infection, viral mRNA is efficiently translated by the existing cellular protein-synthesizing machinery, which appears to be altered in its ability to translate host mRNA.

A similar selective inhibition of host mRNA translation was recently observed when poliovirus-, VSV-, or reovirus-infected cells were exposed to hypertonic growth medium (17) or to other conditions (5) that result in a selective reduction in the overall rate of polypeptide chain initiation (15). This observation has been extended to other RNA and DNA viruses, including rabies virus (10), Semliki forest virus (5), simian virus 40 (4; M. Oppermann and G. Koch, submitted for publication), and vaccinia virus (16). Since the translation of viral mRNA was only slightly affected when the overall rate of polypeptide chain initiation was reduced, it was suggested that viral mRNA possesses a greater intrinsic ability to form functional mRNA-ribosome initiation complexes (15). A theoretical basis consistent with this interpretation is provided by a model proposed by Lodish for the regulation of α- and β-globin mRNA translation (9). Moreover, each mRNA species, viral or cellular, appears to possess an individual rate constant for the initiation of translation (5, 9, 13, 14), which is most probably related to the structural features at the 5'-ends of the mRNAs (2).

In this regard, recent studies have revealed that the translation of individual cellular mRNAs in uninfected cells is also differentially inhibited when the overall rate of polypeptide chain initiation is reduced (5, 14). In MPC-11 mouse plasmacytoma cells, the synthesis of the immunoglobulin G (IgG) light (L) and heavy (H) chains was shown to be 3 to 4 and 1.5 to 2 times more resistant, respectively, to inhi-

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bition by hypertonic initiation block than was the synthesis of non-IgG proteins (14). However, IgG L- and H-chain synthesis was not more resistant than the synthesis of non-IgG proteins when MPC-11 cells were exposed to inhibitors of polypeptide chain elongation.

The observation that host mRNA translation could be selectively reduced by exposure of virus-infected cells to hypertonic conditions prompted us to suggest (15) that the strategy used by some viruses early in the infectious cycle to suppress host mRNA translation might include a reduction in the overall rate of polypeptide chain initiation. Furthermore, it was predicted (13, 15) (i) that such a mechanism would result in a differential suppression in the translation of individual host mRNA species, and (ii) that the differential reduction would be directly related to the intrinsic translational efficiencies of those mRNA species. In other words, the translation of those cellular mRNA species that are most resistant to a reduction in the overall rate of polypeptide chain initiation would also be most resistant to virus-directed suppression.

To test these predictions, the effect of VSV infection on the relative synthesis of the IgG L-chain, IgG H-chain, and non-IgG proteins in MPC-11 cells has been measured. We now report that (i) VSV infection results in a differential suppression of the translation of individual MPC-11 mRNA species and (ii) the translation of those mRNA species (those coding for the IgG proteins) that were previously shown to be most resistant to inhibition after exposure of uninfected cells to hypertonic medium are also most resistant to virus-directed suppression.

MATERIALS AND METHODS

Growth of cells and infection with virus. MPC-11 mouse plasmacytoma cells, originally obtained from Matthew Scharff, Albert Einstein College of Medicine, New York, N.Y., were maintained in Dulbecco modified minimal essential medium (MEM) as described previously (14).

Adsorption of VSV, serotype Indiana, originally obtained from Amiya K. Banerjee, Roche Institute of Molecular Biology, was performed in 250-ml tissue culture flasks (Falcon) at room temperature for 20 min at a cell density of 10^7 cells/ml in serum-free Dulbecco MEM. After adsorption, infected or mock-infected cell suspensions were diluted to a density of 10^6 cells/ml in Dulbecco MEM supplemented with 10% horse serum and incubated in the presence of CO_2 at 37°C. The time of dilution was taken as time zero of the infectious cycle. In all experiments the multiplicity of infection was 20.

Incorporation studies. Samples of infected or mock-infected cells were removed from the respective cultures at appropriate times throughout the infectious cycle, harvested by centrifugation, and resuspended at a cell density of 3×10^6 cells/ml in 3 ml of serum-free Joklik modified MEM–25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.4, containing 1/20 the normal amount of methionine. Each cell suspension was incubated for 15 min at 37°C after the addition of 100 μCi of [^{35}S]methionine (Amersham), at which time 50-μl portions were removed for quantitation of [^{35}S]methionine incorporation into protein according to the method of Mans and Novelli (11). The remainder of each cell suspension was immediately diluted into 40 ml of semifrozen MEM containing a 10^4-fold excess of unlabeled methionine. The cells were pelleted, resuspended in the same medium, and incubated at 37°C for an additional 15 min. After the chase procedure, the cells were washed with phosphate-buffered saline at 4°C, and cytoplasmic extracts were prepared and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (14).

Autoradiographs of the dried slab gels were analyzed and quantitated with an integrating densitometer (Orteck, model 4310). An appropriate exposure time was chosen in which the film gave a linear response to the amount of radioactivity. Alternatively, the radioactivity incorporated into individual polypeptides was measured directly by excising the appropriate bands from the dried gel and dissolving each band in 0.3 ml of 15% hydrogen peroxide, followed by counting in Aquasol liquid scintillation fluid. The two methods yielded comparable results.

The relative number of methionine residues in the IgG L and H chains was determined by measuring the ratio of radioactivity found in the L and H chains derived from [^{35}S]methionine-labeled H_2L_2 molecules recovered from polyacrylamide gels. This value was then used for calculating the relative synthesis of the L and H chains.

RESULTS

Reduction in [^{35}S]methionine incorporation into total protein after virus infection. Within 30 min after infection of MPC-11 cells by VSV, incorporation of [^{35}S]methionine into protein is reduced by 30 to 35% with respect to the level of incorporation in mock-infected cells (Fig. 1). Total incorporation continues to decline, although at a slower rate, as the infectious cycle proceeds, with an increase in total incorporation usually observed between 3 and 4 h postinfection. As will be shown later, the synthesis of virus-specific proteins is maximal during this period. It can be seen from Fig. 1 that the level of [^{35}S]methionine incorporation in mock-infected cells does not decline but increases by approximately 7% by 5 h. The reduction in the total rate of protein synthesis observed over the time course of VSV infection of mouse plasmacytoma cells is similar to that reported by Wertz and Youngner for mouse L cells after infection with VSV (21). In contrast, infection of HeLa cells by VSV does not result in an early

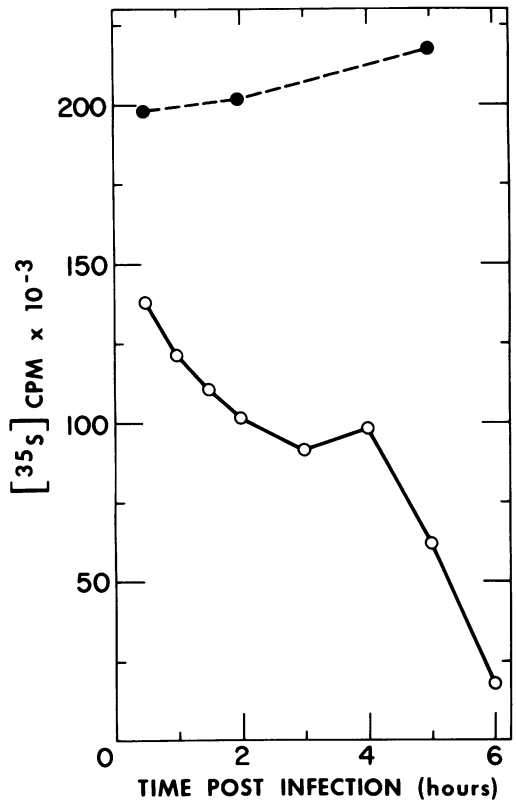

FIG. 1. *[35S]methionine incorporation into total protein in mock-infected and VSV-infected cells. Samples (9 ml) were removed from mock-infected (●) or VSV-infected (○) cultures and pulse labeled for 15 min with [35S]methionine at the time indicated, as described in Materials and Methods.*

reduction in total protein synthesis and is reduced by only 50% as late as 7 h postinfection (15).

Variation in the relative synthesis of host proteins after virus infection. The relative synthesis of virus- and host-specific proteins was determined throughout the infectious cycle by pulse labeling portions of infected or mock-infected cell cultures with [35S]methionine and analyzing the labeled cytoplasmic extracts by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The relative synthesis of the IgG L chain, H chain, and non-IgG proteins was also monitored and compared with the previously determined variations in the relative synthesis of these proteins in uninfected cells under conditions of a reduced rate of either polypeptide chain initiation or elongation (14). A gel autoradiograph obtained from such an experiment is shown in Fig. 2. Visual inspection of the autoradiograph reveals the progressive decrease in the intensity of the host-specific bands and the progressive increase in the intensity of the virus-specific bands, with the peak of virus protein synthesis being observed at 3 h postinfection. The VSV nucleocapsid protein (N) is clearly detectable by 60 min postinfection, whereas the VSV glycoprotein (G), minor protein (NS), and membrane matrix protein (M) are just visible at this time. The VSV large protein (L) becomes detectable by 120 min postinfection. It is also evident from this figure that the intensity of a number of host bands is reduced to different extents after infection, particularly the second major band migrating above the H band.

To estimate the relative synthesis of various host and viral proteins, the autoradiographs were analyzed and quantitated with an integrating densitometer. Presented in Fig. 3 is a comparison of profiles of cytoplasmic extracts from mock-infected (A) and VSV-infected (B) cells pulse labeled 60 min postinfection. Integration of identical regions corresponding to the peaks for the L chain, the H chain, and the remainder of the tracing as a whole in both gel channels revealed that the incorporation of [35S]methionine into the total non-IgG proteins was reduced by 41% after 60 min of infection, whereas the level of incorporation into the H and L chains was reduced by 28 and 7%, respectively. Consequently, the ratio of L synthesis to H synthesis, after correction for the relative number of methionine residues in the two chains, increased from a value of 1.7 in the mock-infected cells to a value of 2.4 in the VSV-infected cells. Moreover, the percentage of the total [35S]methionine incorporation found in the L and H chains increased from estimated values of 8.8 to 13.8% and 9.5 to 11.6%, respectively. Although these alterations in the relative synthesis of MPC-11 proteins occur within 30 min postinfection, when no virus protein synthesis is detectable, the profiles from cells pulse labeled at 60 min postinfection are presented to show that a productive infection has been initiated, as indicated by the emergence of the peak corresponding to the N protein. However, viral protein synthesis still represents less than 5% of the total protein synthesis at 60 min postinfection.

A more direct estimate of the relative synthesis of individual host and viral polypeptides can be obtained by excising specific host and viral bands and measuring the radioactivity. First, the bands corresponding to the L- and H-chain and the five viral polypeptides were carefully excised from the dried slab gels. Then, to obtain a value for the nonviral, non-IgG proteins, 0.5-cm fractions were removed from the remainder of each gel channel, and each band or fraction

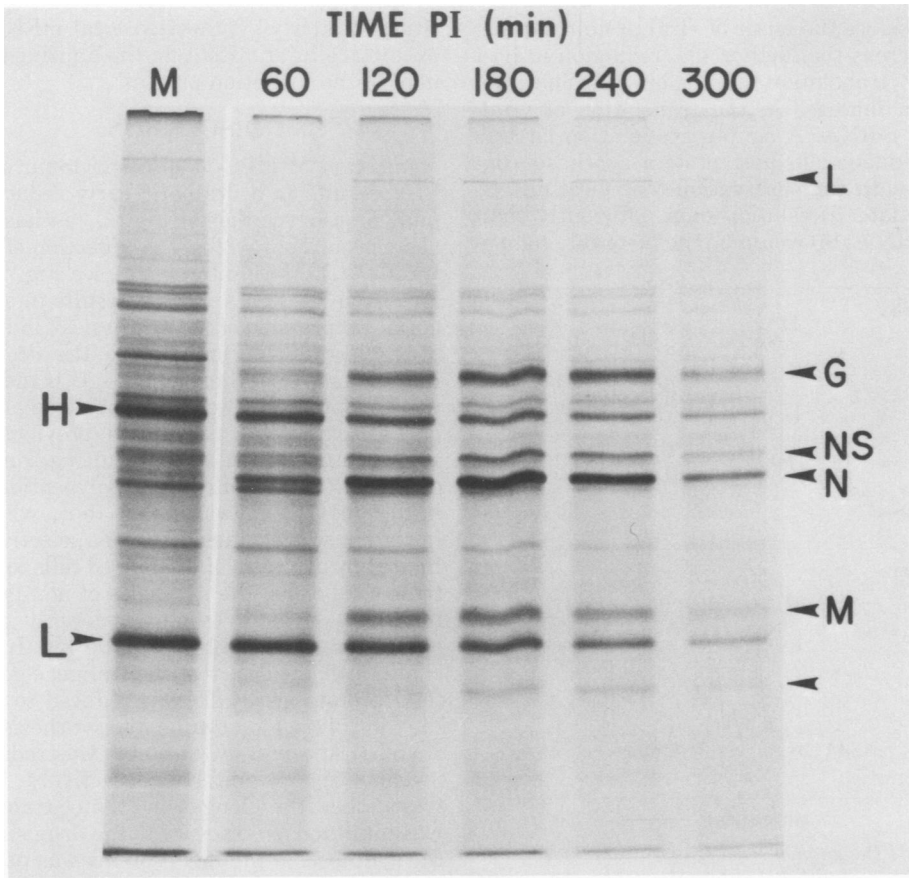

FIG. 2. *Autoradiograph of sodium dodecyl sulfate-polyacrylamide gel analyzing [^{35}S]methionine-labeled cytoplasmic extracts of mock- and virus-infected cells. The arrows at the left of the figure indicate the migration positions of the immunoglobulin L and H chains, whereas the arrows on the right designate the position of the five polypeptides encoded by the VSV genome (3, 12, 19), including the large protein (L), the glycoprotein (G), the minor protein (NS), the nucleocapsid protein (N), and the membrane matrix protein (M). The arrow without accompanying notation designates the position of an unidentified low-molecular-weight (less than 20,000), virus-induced or virus-specific polypeptide that is routinely observed only late in the infectious cycle. The numbers at the top of each gel channel signify the time postinfection at which pulse labeling was performed, and the channel designated M contains the cytoplasmic extract from mock-infected cells.*

was dissolved in 15% hydrogen peroxide and counted in Aquasol counting fluid. The results (Table 1) are in agreement with the conclusions drawn from inspection of Fig. 2 and 3; e.g., the incorporation of [^{35}S]methionine into the L chain was found to be reduced by 12%, whereas the level of incorporation into the H chain and the non-IgG proteins as a whole was reduced by 34 and 51%, respectively, by 60 min postinfection. This difference in the relative reduction in the synthesis of the three protein categories is observed throughout the infectious cycle, although the magnitude of the differences decreases at later times in the infection. Consequently, the relative synthesis of L to H increases progressively from 1.7 to 2.6 by 3 h and remains constant or slightly decreases thereafter.

Relative synthesis of host-specific and virus-specific proteins during the infectious cycle. The relative changes in the synthesis of viral and total nonviral proteins throughout a typical infectious cycle was also determined from gels, as described for Table 1, and are presented in Fig. 4. There is a biphasic reduction in the synthesis of host proteins, with a rapid decrease of approximately 40% within the first 60 min and a further reduction of 40 to 50% over the remaining 4 h. As previously stated, the early reduction in host protein synthesis

occurs before the onset of viral protein synthesis, whereas the further 40% reduction in host mRNA translation proceeds concomitantly with an increase in the translation of viral-specific mRNA. A simple explanation for this later reduction in host protein synthesis consistent with the observation that viral mRNA can initiate translation more efficiently than host mRNA (15) would be the favorable competition by newly synthesized viral mRNA with existing cellular mRNA for the limiting constituent of the initiation process.

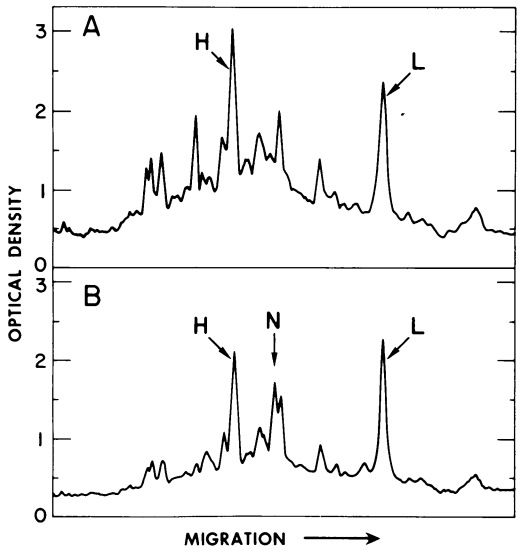

FIG. 3. *Densitomer tracing of sodium dodecyl sulfate-polyacrylamide gel autoradiographs. An autoradiograph of sodium dodecyl sulfate-polyacrylamide gels analyzing cytoplasmic extract of mock-infected (A) and VSV-infected (B) cells pulse labeled with [^{35}S]methionine 60 min postinfection was analyzed by an Orteck integrating densitometer. The arrows designate the position of the IgG L chain, the IgG H chain, and the VSV nucleocapsid protein (N).*

DISCUSSION

Infection of MPC-11 plasmacytoma cells by VSV results in a dramatic early reduction in total protein synthesis (Fig. 1), as has previously been reported for VSV infection of mouse L cells (21). More interesting (see Fig. 2 and 3 and Table 1), VSV infection results in a differential suppression of the synthesis of individual MPC-11 proteins. Furthermore, the alterations in the relative synthesis of the IgG L and H and non-IgG proteins after VSV infection are comparable with the alterations previously observed in uninfected cells, resulting from a reduction in the overall rate of polypeptide chain initiation (14). It was found that, when the initiation step of translation was selectively inhibited by exposure of uninfected cells to hypertonic conditions, the synthesis of non-IgG proteins was reduced to a considerably greater extent than the synthesis of the IgG L and H chains over the dose response range; e.g., when total protein synthesis was reduced to 11% of the control (isotonic) value, the synthesis of IgG L and H and non-IgG proteins was reduced to levels of 29, 15, and 9%, respectively. In contrast, when the elongation step of translation was inhibited by exposure to the drug emetine, the synthesis of the IgG proteins was not more resistant than the synthesis of non-IgG proteins; e.g., when total protein synthesis was inhibited by 75%, the synthesis of L, H, and non-IgG proteins was reduced to levels of 26, 20, and 26%, respectively. Furthermore, it was clearly shown (14) that a value for the ratio of L- to H-chain synthesis (L/H) in excess of 2 was

TABLE 1. *Relative Synthesis of L, H, and non-IgG proteins after infection of MPC-11 cells by VSV*[a]

Time post-infection (min)	[^{35}C]methionine incorporation (cpm × 10^{-3})						L/H
	L	% of control	H	% of control	Non-IgG	% of control	
Mock	9.3	(100)	11.7	(100)	144	(100)	1.7
60	8.1	(88)	7.7	(66)	71	(49)	2.3
120	6.1	(66)	5.6	(48)	52	(36)	2.4
180	5.2	(56)	4.4	(38)	44	(31)	2.6
240	3.7	(41)	3.4	(29)	31	(22)	2.4
300	2.3	(25)	2.0	(17)	21	(15)	2.5

[a] The bands corresponding to the IgG L chain, IgG H chain, and VSV proteins, were excised, dissolved in 15% hydrogen peroxide, and counted in Aquasol scintillation fluid. The remainder of each gel channel was then excised in 0.5-cm fractions and subjected to the same process and is reported as non-IgG proteins. The numbers in the columns designated by L, H, and non-IgG represent ^{35}S counts per minute recovered in the respective gel regions. The numbers in parentheses represent the remaining level of synthesis presented as the percentage of the level observed in the mock-infected cells pulse labeled at 60 min postinfection. Also included is the value for the ratio of L to H synthesis after infection. This value was obtained by correcting for the relative number of methionine residues in the L and H chains, which was determined by measuring the ratio of radioactivity in L and H derived from H_2L_2 molecules recovered from polyacrylamide gels (13).

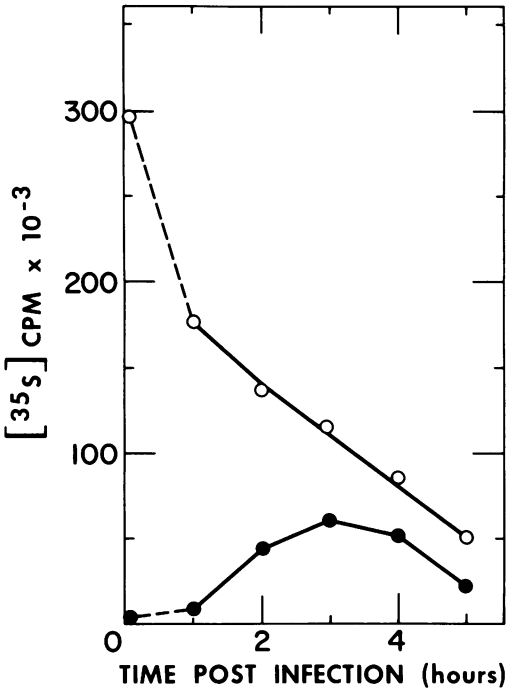

FIG. 4. *Relative synthesis of host- and VSV-specific proteins after infection. The incorporation of [^{35}S]methionine into the five VSV polypeptides (●) and total non-VSV proteins (○) was determined as described for Table 1. The values indicated at 0 time were obtained from mock-infected cells pulse labeled at 60 min postinfection. The dashed line joining the 0- and 60-min time points are included to emphasize this point.*

indicative only of a sitiation in which the rate of initiation had been selectively inhibited.

Thus, the relative sensitivities of IgG and non-IgG protein synthesis observed in Fig. 2 and 3 and Table 1, as well as the increased (L/H) value, are consistent with an early virus-induced reduction in the overall rate of polypeptide chain initiation. Although the exact mechanism whereby VSV infection results in this early suppression is unclear, it is known that the initiation of viral mRNA translation can proceed at high rates under other experimental conditions (e.g., exposure of cells to hypertonic medium), which also results in alterations in the synthesis of MPC-11 proteins similar to those observed in Fig. 2 and 3 and Table 1 (14, 15). This is not to say that virus-directed suppression of host translation and inhibition of protein synthesis by hypertonic conditions necessarily proceed via the same mechanism, but that the final result on the relative synthesis of the individual cellular proteins is essentially the same in both cases.

The initial reduction in host protein synthesis of roughly 30 to 40% discussed above (Fig. 4) is followed by a further 40 to 50% reduction concomitant with an increase in viral protein synthesis and presumably an increase in the concentration of viral-specific mRNA in the cell. Since viral mRNA possesses a higher rate constant for the initiation of protein synthesis (15), an increase in the concentration of viral mRNA later in the infectious cycle would result in a further reduction in host mRNA translation due to a favorable competition by viral mRNA for the limiting constituent involved in the initiation process.

The selective inhibition of host mRNA translation in virus-infected cells exposed to hypertonic medium (15), the differential inhibition of host mRNA translation by VSV infection, and the similarity in the alterations in the relative synthesis of MPC-11 proteins by VSV infection and by hypertonic initiation block (14) have led us to conclude that VSV-directed suppression of host mRNA translation can be understood in the following terms: (i) a relatively higher rate constant for polypeptide chain initiation possessed by viral mRNA; (ii) an early virus-induced reduction in the overall rate of polypeptide chain initiation and the relative effect this event would have on the translation of host and viral mRNA; and (iii) a favorable competition by viral mRNA with host mRNA for the formation of functional mRNA-ribosome initiation complexes as the concentration of viral mRNA increases.

Although this report deals only with VSV-directed suppression of host translation, it seems likely that the results presented here pertain, in a general way, to the shut-off strategy of other animal viruses. Recent studies investigating the effect of poliovirus infection on the relative synthesis of several individual proteins in BSC-1 cells (5; H. Oppermann and G. Koch, unpublished data) support this conclusion.

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