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Transcription of Vesicular Stomatitis Virus Is Required to Shut Off Cellular RNA Synthesis

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RNA synthesis by mouse myeloma (MPC-11) cells was rapidly and progressively shut off by infection with vesicular stomatitis virus temperature-sensitive (*ts*) mutants permissive for transcription. In sharp contrast, mutants or defective vesicular stomatitis virions restricted in transcription were incapable of causing progressive inhibition of cellular RNA synthesis even at massive multiplicities of infection. A viral product synthesized 30 to 60 min after permissive infection with *ts*G114(I) appeared to be essential for prolonged inhibition of RNA synthesis in cells switched up to restrictive temperature.

The killing of cells and the inhibition of host RNA (1, 21, 23) and protein (10–12, 14, 22) synthesis by vesicular stomatitis (VS) virus apparently require the production of certain functional viral components, but none have yet been identified. Since previous work in this laboratory revealed that mouse myeloma (MPC-11) cellular RNA polymerase activity was quite susceptible to shut-off by VS virus (21), we have sought to determine which viral function might be involved in this inhibition. It has been suggested that there are two possible mechanisms responsible for the inhibition of host protein synthesis by VS virus (22). One is a multiplicity-dependent effect that results in an initial drop in cellular activity, and the second is progressive and requires the synthesis of viral mRNA and/or polypeptides following infection (10, 13). In an effort to test whether these mechanisms might also function to alter host RNA metabolism (21), MPC-11 cells were tested for their response to infection with various VS virus temperature-sensitive (*ts*) mutants restricted in different functions. In addition, we examined the effect on cellular RNA metabolism of defective-interfering particles which cannot synthesize mRNA or proteins owing to massive deletion of the genome. Since the defective particles employed in these experiments are known to transcribe only a short sequence from the 3′ terminus of the deleted viral genome (3, 18), we undertook to examine what role partial transcription of the genome might play in the alteration of cellular RNA biosynthesis.

The growth and maintenance of mouse myeloma (MPC-11) cells, the production of wild-type VS virus (Indiana serotype, San Juan strain), and the measurement of RNA synthesis in the myeloma cells were performed as previously described (21). Two RNA$^-$ *ts* mutants of VS virus employed in these experiments were provided by C. R. Pringle, Institute of Virology, Glasgow, Scotland (16); the mutant from complementation group I (*ts*G114) is defective in primary and secondary transcription (5, 9, 15), and the group IV mutant (*ts*G44) fails to replicate but can carry out primary transcription at the nonpermissive temperature (2, 19, 20). Mutants *ts*O52(II) and *ts*O23(III) were kindly provided by A. Flamand and F. Lafay, Faculté des Sciences, Université de Paris-Sud, Orsay, France (4, 6). All the mutants were grown on BHK-21 cells and titrated by assay of PFU on monolayers of L cells. Defective-interfering particles were produced by undiluted passage on BHK-21 cells from stock preparations obtained from S. U. Emerson of our department. The particles were purified by a series of sucrose gradient centrifugations and determined to be free of plaque-forming virus by plating on monolayers of L cells (3).

Previous work had demonstrated that wild-type VS virus almost completely inhibits incorporation of [^{3}H]uridine into rapidly synthesized RNA in MPC-11 cells by 4 h postinfection and causes a significant decline in RNA metabolism of host nuclei measured in vitro (21). In an attempt to determine what viral functions are responsible for this inhibition, *ts* mutants of VS virus from complementation groups I, II, and IV were tested at restrictive temperature for their ability to alter host RNA synthesis.

Figure 1 compares the capability of the different mutants to inhibit incorporation of [^{3}H]uridine into cellular RNA products at the nonpermissive temperature of 39°C. Only *ts*G114(I) restricted in transcriptional activities failed to shut off host RNA metabolism when incubated

at 39°C for 4 h. Although mutant *ts*G44(IV) did not appear to inhibit cell RNA synthesis as rapidly as *ts*O52(II), extension of incubation time for an additional hour resulted in almost identical values of RNA inhibition for the wild-type, *ts*O52(II), and *ts*G44(IV) infected cultures (data not shown).

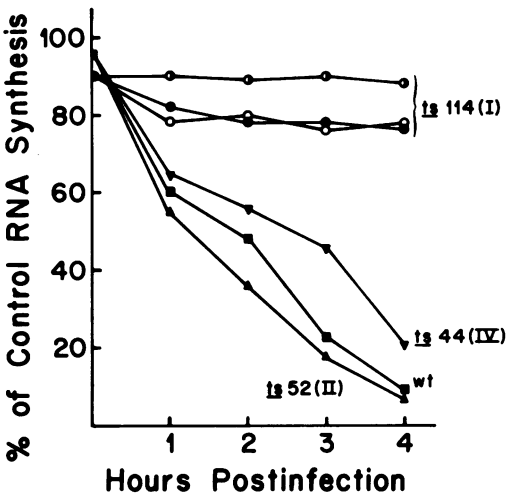

FIG. 1. *Inhibition of RNA synthesis in MPC-11 cells following infection with ts mutants of VS virus at 39°C. Mouse myeloma cells were infected with wild type (WT) (■), tsG44(IV) (▼), or tsO52(II) (▲) of VS virus at a multiplicity of infection of 20 PFU/cell. In a similar experiment, cells were infected with tsG114(I) at a multiplicity of 1 (◑), 20 (●), or 200 (○) PFU/cell. Following infection, the cells were maintained at 39°C, and cell RNA synthesis was measured by pulse-labeling the cells for 15 min in medium containing 2 μCi of [³H]uridine per ml (26 Ci/mmol) at hourly intervals postinfection. The data are presented as the percentage of acid-insoluble RNA synthesized by mock-infected cells maintained under the same conditions. Incorporation of [³H]uridine by control cells ranged from 6.2 to 6.6 pmol/mg of protein.*

It has been postulated that VS viral inhibition of cellular macromolecular synthesis is primarily due to perturbation of host cell plasma membrane by a structural component of the invading virion (1). This hypothesis can be tested with mutant *ts*G114(I) since it synthesizes no viral mRNA or protein in L cells infected at 39°C (10) or MPC-11 cells infected at 39°C (data not shown). As depicted in Fig. 1, *ts*G114(I) caused only ~10% reduction in cell RNA synthesis at a multiplicity of infection of 1 and only about 20% loss at multiplicities of 20 or 200. These results indicate that, although there is an initial minor decline in cellular RNA synthesis, the absorption of large quantities of metabolically inert virus to the cell surface does not in itself produce the drastic reduction in cellular synthesis caused by VS virus. This observation is consistent with the results reported by Wertz and Youngner (22), who were able to detect a slight drop in cell protein synthesis immediately after adsorption to L cells of UV-irradiated VS virus.

The inability of *ts*G114(I) to inhibit cellular RNA synthesis at 39°C suggested that primary transcription of the viral genome is essential to compromise host RNA as well as protein (10) synthesis. However, proof was required that active transcription by *ts*G114(I) at permissive temperature would result in inhibition of cellular RNA synthesis comparable to that of wild-type and other *ts* mutants. The results (Table 1) demonstrate that mutants from groups I through IV are all capable of shutting off cellular RNA synthesis at 31°C. Although inhibition of RNA synthesis by mutants at this temperature was not as marked as that at 39°C, shut-off was as complete as that produced by wild-type infection at the permissive temperature (Table 1). These temperature differences in the degree of inhibition are almost surely due to lower incor-

TABLE 1. *Effects of wild-type and temperature-sensitive mutants of VS virus on MPC-11 cellular RNA synthesis at 31 and 39°C*[a]

Time post-infection (h)	RNA synthesis[b]									
	Wild type		*ts*G114 (I)		*ts*O52 (II)		*ts*O23 (III)		*ts*G44 (IV)	
	31°C	39°C	31°C	39°C	31°C	39°C	31°C	39°C	31°C	39°C
0	98	97	97	90	94	90	94	98	92	91
1	65	76	91	83	87	56	63	56	76	66
2	53	48	62	77	55	36	49	36	71	57
3	46	22	55	77	57	18	46	16	63	44
4	39	7	35	80	36	6	41	6	50	19

[a] MPC-11 cells were infected at an input multiplicity of 20 PFU per cell for 30 min at 23°C. Immediately after adsorption, the cells were pelleted and suspended in media prewarmed to 31 or 39°C and incubated in 10-ml spinner cultures. At various times postinfection, samples of cells were removed and pulse-labeled, and the amount of radioactive acid-insoluble RNA product was determined as previously described (21).

[b] Values expressed as the percent RNA synthesis in mock-infected cells incubated at the appropriate temperature.

poration of [^{3}H]uridine, reflecting a reduction in overall cellular metabolism at 31°C. Incubations extended to 6 h postinfection resulted in 90% inhibition of RNA synthesis (data not shown). Therefore, it appears that *ts*G114(I) must undergo primary transcription and possibly translation to exhibit a progressive inhibitory effect on cellular RNA synthesis.

Additional evidence for the requirement of a critical transcriptional event was sought by temperature shift-up experiments. MPC-11 cells infected with *ts*G114(I) were incubated for brief periods at permissive temperature to initiate viral RNA and protein synthesis before shifting to the restrictive temperature. When cells infected with *ts*G114(I) were first incubated at 31°C and subsequently shifted to 39°C, there appeared to be a critical period of time required for the production of the inhibitory component (Fig. 2). If infected cells were incubated for 30 min or less, there was only a 20 to 25% decrease in cellular RNA metabolism which did not change during incubation at 39°C for 4.5 h. However, upon extension of the initial incubation to 60 min, there was a rapid decline in the ability of cells to synthesize RNA following the shift-up to 39°C (Fig. 2). In fact, the degree to which cellular RNA synthesis was inhibited during the second incubation period was significantly greater than that observed in *ts*G114-infected cells maintained at 31°C for the entire period of 5 h (Fig. 2). These results reveal that some viral product must be made before maximum RNA shut-off of the cell can occur; once the mutant viral function is expressed, an increase in temperature does not reverse the process but even tends to hasten the inhibition of cellular RNA synthesis.

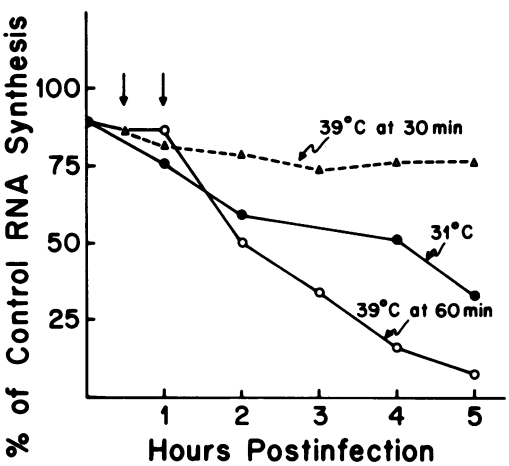

FIG. 2. *Effect of temperature shift on the rate of inhibition of RNA synthesis in cells infected with mutant tsG114(I). MPC-11 cells were infected (multiplicity of 20 PFU per cell) at 23°C and maintained at either the permissive temperature of 31°C (●) or shifted (arrows) from 31°C to the nonpermissive temperature of 39°C at 30 min (△) or 60 min (○) following infection. At hourly intervals, cells were pulse-labeled for 15 min in media containing [^{3}H]uridine, and the incorporation of radioactivity in acid-insoluble material was determined. The data are expressed as the percentage of RNA synthesis in mock-infected cells incubated under comparable conditions.*

The propagation of VS virus at high multiplicity results in the production of virus particles that are smaller and contain shorter genomes than the normal VS virus particle (8, 17). Since such particles fail to replicate alone, these T particles provide a means of examining the effects of a nonreplicating truncated particle on cellular RNA metabolism. Until recently, no transcriptional products had been found to be synthesized by purified T particles in which the 3′ end of the genome was deleted. However, it is now evident that certain defective particles synthesize a unique leader RNA sequence of ~45 nucleotides in length (3, 18). If the transcription of this short sequence is involved in shutting off host RNA metabolism, then infection of cells with purified T particles should result in a reduction of the incorporation of [^{3}H]uridine into acid-insoluble cellular RNA products. When MPC-11 cells were infected with purified particles, derived from the 5′ end of the genome but designated LT (3), there was no significant reduction of host cell RNA synthesis (Table 2).

TABLE 2. *Effect of defective-interfering particles of VS virus on cellular RNA synthesis at various multiplicities of infection*[a]

Time postinfection (h)	% RNA synthesis by MPC-11 cells at the following multiplicities of infection:[b]			
	100	500	1,000	10,000
0	98	100	88	85
1	97	100	79	91
2	92	104	84	88
3	94	97	72	83
4	98	87	74	86

[a] Following adsorption of defective-interfering particles for 30 min at 23°C, MPC-11 cells were incubated at 37°C in 10-ml spinner cultures. At the times indicated, samples of infected or mock-infected cells were pulse-labeled with [^{3}H]uridine for 10 min at 37°C, and the amount of trichloroacetic acid-precipitable material was determined as described previously (21).

[b] Values indicate percent incorporation of [^{3}H]uridine by mock-infected cells and represent the average values of two independent experiments. The multiplicity of infection is based on the amount of protein in the defective-interfering preparation and is expressed as the number of particles per cell.

Even at a multiplicity as high as 10,000 particles per cell, there appeared to be only a slight reduction (~10 to 15%) in RNA synthesis. Thus, even if the very short leader sequences are transcribed in vivo by this type of T particle, it appears that they play no role in inhibition of cellular RNA polymerase activity. Recent evidence indicates that only LT particles, derived from the 3′ end of the genome and capable of transcribing N, NS, M, and G genes, can kill cells, and that short defective-interfering particles are incapable of cell killing (13). Previous reports concerning shut-off of RNA synthesis by metabolically inactive short defective-interfering particles (1, 8) might be explained by the fact that earlier investigations were performed with a different class of defective particle or could be contaminated with infectious B particles.

The results presented in this paper indicate that certain viral products must be made before drastic and prolonged shut-off of host RNA metabolism. At the present time, it is not known whether this product is transcriptional or translational in nature because all attempts to block viral protein synthesis by inhibitors, such as cycloheximide or puromycin, resulted in marked inhibition of cellular RNA synthesis (unpublished data of P. K. Weck). Cycloheximide has been found to inhibit DNA-dependent RNA polymerases in eucaryotic cells (7). Although the defective-interfering particle product alone cannot shut off the cell, it is still possible that a leader RNA sequence transcribed from another region of the intact genome is all that is required to inhibit host RNA synthesis. This question of whether transcription of the viral genome alone is sufficient to cause a decrease in host cellular RNA synthesis is presently being investigated.

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