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ARTICLE *in* BIOCHEMISTRY · MARCH 1980

Impact Factor: 3.02 · DOI: 10.1021/bi00545a029 · Source: PubMed

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Inhibition of Ribonucleic Acid Accumulation in Mouse L Cells Infected with Vesicular Stomatitis Virus Requires Viral Ribonucleic Acid Transcription†

Fang-Sheng Wu and Jean M. Lucas-Lenard*

ABSTRACT: The accumulation of ribonucleic acid (RNA) in mouse L-929 cells infected with temperature-sensitive mutants of vesicular stomatitis virus or ultraviolet- (UV-) irradiated virus was studied. At the permissive temperature (30 °C) infection by all mutants resulted in an inhibition of cellular RNA accumulation. At the nonpermissive temperature (40 °C) mutants G114 (I) and G22 (II) failed to inhibit RNA accumulation, but mutants G11 (I), O52 (II), G31 (III), G33 (III), G41 (IV), W10 (IV), O45 (V), and O110 (V) were still active in this respect. In most cases the accumulation of 28S and 18S mature rRNA was inhibited to a greater extent than the synthesis of the 45S rRNA precursor. UV irradiation of wild type virus considerably reduced its capacity to inhibit cellular RNA synthesis. The target size for inactivation of this capacity of the virus was ~17% of the viral genome or that corresponding to the N gene. These results indicate that the virion proteins themselves are incapable of inhibiting cellular RNA synthesis and that transcription of ~17% of the genome is required. Expression of RNA synthesis inhibition also requires some function of virion NS protein in addition to its transcriptase activity.

Infection of vertebrate cells with vesicular stomatitis virus (VS virus) causes a marked inhibition of synthesis of cellular macromolecules (Wagner, 1975). Previous reports have suggested that cellular protein synthesis inhibition in L cells infected by VS virus requires virion-associated L protein and transcription and translation of viral genes N and possibly NS (Marvaldi et al., 1977, 1978). The mechanism of cellular RNA synthesis inhibition is not understood. High multiplicities of input intact virions (Huang & Wagner, 1965), defective interfering particles (Baxt & Bablanian, 1976), and large amounts of isolated G (glyco) protein (McSharry & Choppin, 1978) have been reported to inhibit cellular RNA synthesis. The inhibition of RNA synthesis in chicken embryo cells after infection with VS virus is partially a result of a decreased capacity of the infected cells to transport uridine (Genty, 1975). However, in infected L cells, HeLa cells (Genty, 1975), and mouse myeloma cells (Weck & Wagner, 1978), uridine transport remains unaltered.

Protein synthesis inhibition and cell killing appear to be governed by the same gene functions (Marcus & Sekellick, 1975; Marvaldi et al., 1977). We wished to know whether RNA synthesis inhibition is under the control of the same gene functions as protein synthesis inhibition and cell killing. Temperature-sensitive (ts) mutants which are defective in specific gene functions at the restrictive temperature were screened for their ability to inhibit cellular rRNA synthesis at the nonpermissive temperature to investigate this problem. Mutants unable to inhibit cellular rRNA synthesis at the restrictive temperature were considered to be defective in a function needed for the inhibition. Ultraviolet-irradiated wild type VS virus was also used to determine if newly synthesized viral gene products are necessary or whether virion proteins will suffice. The extent of UV irradiation damage was monitored by protein analysis of the residual transcription and translation of the viral genome (Ball & White, 1976; Marvaldi et al., 1978).

Our results suggest that the virion proteins themselves are not capable of inhibiting cellular RNA synthesis. Transcription of at least part of the viral genome is necessary for the inhibition. While our work was in progress, Weck & Wagner (1979) reported the necessity of viral RNA transcription for cellular RNA synthesis inhibition.

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0006-2960/80/0419-0804$01.00/0

Materials and Methods

Cell Culture. Monolayers of mouse L-929 cells were cultivated in Eagle's minimum essential medium (MEM) supplemented with 8.5% fetal bovine serum (complete medium). Subconfluent monolayers on 100-mm plastic petri dishes (Falcon) containing $\sim 2 \times 10^7$ cells were used in all experiments.

Virus Stock. The heat-resistant (HR) strain of Indiana serotype (Holloway et al., 1970) and the following temperature-sensitive (ts) mutants of VS virus were used: ts G114 (I), G11 (I), G22 (II), G31 (III), G33 (III), and G41 (IV) carrying the G (Glasgow) designation (Pringle, 1970); W10 (IV) carrying the W (Winnipeg) designation (Holloway et al., 1970); O52 (II), O45 (V), and O110 (V) with the O (Orsay) designation (Flamand, 1970; Lafay, 1974). The procedures for the preparation of virus stocks and plaque assays were as described previously (Marvaldi et al., 1977). At the end of each experiment the titer of the virus stock used was checked by plaque assay. For some experiments the stock virus was purified by sucrose gradient centrifugation. Stock virus was layered onto a 15–35% sucrose gradient in buffer containing 0.1 M NaCl, 0.01 M Tris-HCl, pH 7.4, and 0.001 M EDTA. The virus was centrifuged for 2 h at 57700*g* in a Beckman SW 27.1 rotor. The B particles (fastest sedimenting) were removed with a syringe and concentrated by pelleting in a 60 Ti rotor for 3.5 h at 72100*g*. All mutant virus stocks contained less than 0.003% revertants except O52 (II) and G33 (III), which had 0.08 and 0.4% revertants, respectively.

Infection of Cells. Cells were prefed with fresh complete medium for 2 h. After 2 h, they were washed twice with 5 mL of prewarmed phosphate-buffered saline (PBS). Virus stocks were diluted with PBS and added to the monolayers in a volume of 0.6 mL to reach the desired multiplicity of infection (MOI). After an adsorption period of 35 min at 40 °C for experiments at the nonpermissive temperature or 50 min at 30 °C for experiments at the permissive temperature, unadsorbed inoculum was removed, 5 mL of prewarmed complete medium was added to the monolayers, and the monolayers were incubated at the appropriate temperatures.

Labeling and Extraction of RNA. At intervals after infection, the incubation medium was replaced with prewarmed (30 or 40 °C) complete medium containing 25 μCi/mL [5,6-$^3H_2$]uridine (40–50 Ci/mmol). The incubation was continued as indicated in the figure or table legends. After the labeling period, monolayers were cooled in an ice bath and washed 3 times with 5 mL of prechilled PBS. The monolayers were then frozen and stored at −70 °C until the RNA extraction was performed. Usually the storage was less than 5 days.

The cells on the monolayers were lysed with 5 mL of sodium dodecyl sulfate ($NaDodSO_4$) buffer (pH 7.2) containing 0.02 M Tris-HCl, 0.1 M NaCl, 0.005 M EDTA, 0.5% $NaDodSO_4$, and 100 μg/mL protease (type V, Sigma Chemicals) per plate and collected into 50-mL test tubes. Each sample was a combination of two plates. After 30 min at room temperature the lysates (10 mL/sample) were extracted with 18 mL of a 1:1 (v/v) phenol–chloroform mixture which was preheated for 5 min in an 80 °C water bath. The phenol was redistilled and water-saturated prior to use and contained 0.1% of 8-hydroxyquinoline and 10% *m*-cresol. The chloroform in the mixture contained 1% isoamyl alcohol. The extraction was performed by vortexing the mixture in a 50-mL test tube with a cap for 90 s and cooling rapidly in an ice bath. After being cooled, the mixtures were again vortexed for 1 min and then centrifuged at 12000*g* for 15 min at 4 °C. The aqueous phase was reextracted twice with the above cold phenol mixture. To the final aqueous phase was added 1/10 volume of 2.5 M ammonium acetate, and the nucleic acids precipitated with 2 volumes of prechilled (−20 °C) 95% ethanol. After being allowed to stand overnight at −20 °C, the nucleic acid precipitates were collected by centrifugation at 12000*g* for 40 min at −5 °C, dried briefly in N_2 gas, and resuspended in electrophoresis buffer as stated below. DNase (free of RNase activity) and $MgCl_2$ were added to a final concentration of 30 μg/mL and 0.01 M, respectively.

The DNase digestion was carried out at 35 °C for 30 min. The reaction mixture was then deproteinized with cold phenol as described above. The RNA collected after ethanol precipitation was resuspended in electrophoresis buffer (Tris-HCl, 0.04 M; sodium acetate, 0.02 M; Na-EDTA, 0.002 M, pH 7.8) and quantitated by UV absorbance at 260 nm by assuming $E_{260}^{0.1\%} = 24$. The ratio of 260/280 nm of the extracted RNA was 2.0 ± 0.2. For determination of the specific radioactivity of the extracted RNA, the resuspended RNA was precipitated with 10% trichloroacetic acid (Cl_3AcOH) with crude yeast RNA (200 μg/mL) as a carrier and filtered through GF/C glass fiber filter papers with two washes of 10% Cl_3AcOH and 95% ethanol. The filter papers were digested with NCS (Amersham Corp.) solubilizer at 55 °C for 3 h, and radioactivity was determined in 10 mL of toluene containing 4 g/L of 98% PPO and 2% bis(MSB), preblended.

In some cases, the RNA solution was simply diluted with water and counted in a Triton X-100 scintillation fluid containing 66% (v/v) xylene, 34% (v/v) Triton X-100, and 0.8% (w/v) preblended scintillation fluor. By this direct counting method, the counting efficiency was less than that obtained by a Cl_3AcOH–filter paper technique, but the relative ratio between the samples was approximately the same.

Polyacrylamide Gel Electrophoresis. Basically, the method used was that of Loening (1967) modified for the analysis of high molecular weight RNA. One percent agarose was added to the 2% polyacrylamide gels prepared in a vertical slab gel electrophoresis apparatus with 3-mm thickness spacers to reinforce the strength of the gel. Each RNA solution containing 5% sucrose was applied to each well in a volume of 30–50 μL to give the same quantity of RNA among the samples. Electrophoresis was performed at 140 V until the tracking dye (bromophenol blue) ran near the end of the gel. The slab gels were then fixed and stained in 0.5 M acetic acid containing 0.01% methylene blue for 2 h and destained in 0.5 M acetic acid for several hours. The use of a low concentration of stain allowed the fast localization of major RNA bands within 1 h and shortened the destaining time considerably.

Fluorography. The procedure for the fluorography of the acrylamide–agarose composite gels was basically that of Laskey & Mills (1975). The gels were dried under vacuum with heat and then exposed to Kodak X-Omat film at −70 °C. The film was presensitized to a background absorbance of 0.1 before use. The fluorograms were scanned in a Joyce-Loebl densitometer.

UV Irradiation. HR-VS virus preparations were irradiated with UV light at a wavelength of 254 nm as described previously (Marcus & Sekellick, 1975), under conditions which produced a dose rate of 65.4 ergs/(mm^2 10 s).

Incorporation of [^3H]Uridine into Acid-Soluble and Acid-Insoluble Material. Monolayers were infected with HR-VS virus at a MOI of 50 at either 30 or 40 °C. When the experiment was performed at 30 °C, the monolayers were preincubated at 30 °C for 18 h before infection to stabilize

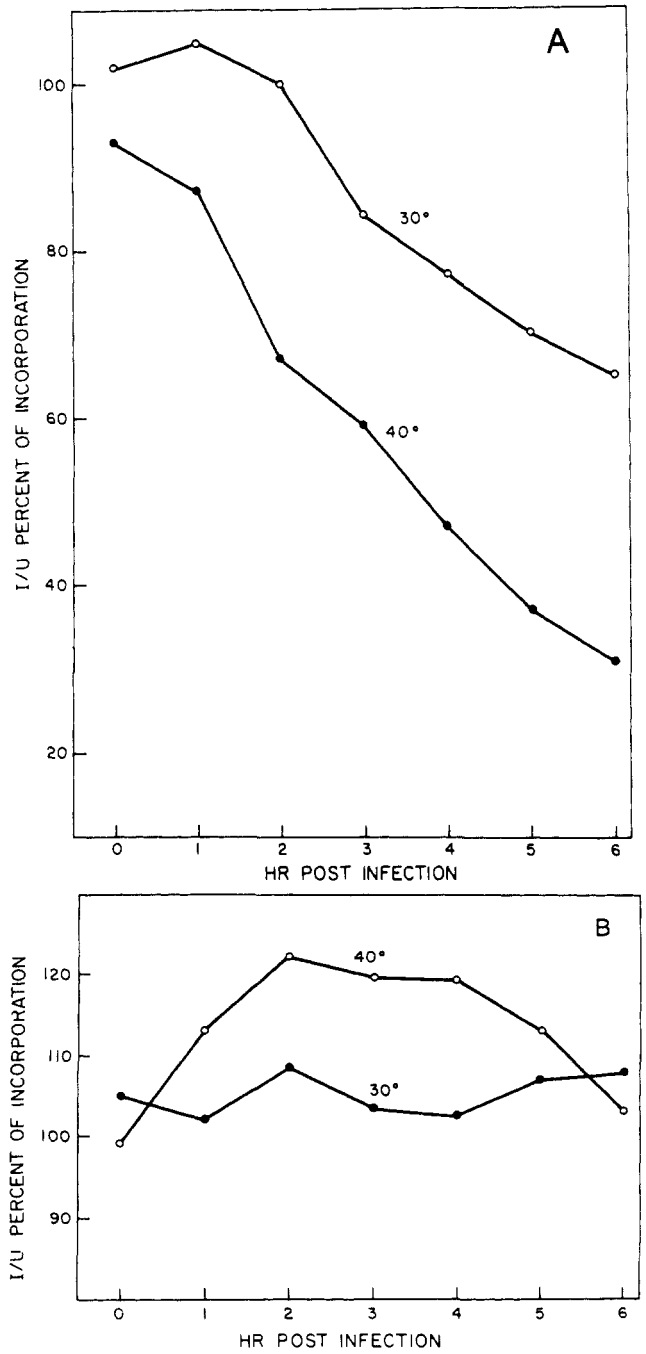

FIGURE 1: [^{3}H]Uridine incorporation into acid-insoluble (A) and acid-soluble (B) materials of mouse L cells infected with wild type VS virus. Cells were infected at an MOI of 50 and incubated at 30 or 40 °C. At the time points indicated in the figure, the cells were pulsed with [^{3}H]uridine for 30 min and treated as described under Materials and Methods. Each time point represents an average of two experimental values.

the growth condition of the cells. At different times after infection, the medium was replaced with prewarmed (30 or 40 °C) complete medium containing 10 μCi/mL [^{3}H]uridine. After pulse-labeling for 30 min, the monolayers were cooled on ice and washed 3 times with 5 mL of prechilled PBS. For determination of the amount of [^{3}H]uridine incorporated into acid-soluble pools, 3 mL of 7% Cl_3AcOH was added onto each monolayer and incubated on ice for 10 min. The extraction was repeated once, and the extracts were combined. The radioactivity was determined by taking aliquots from the combined extracts and mixing them with 10 mL of a Triton X-100–xylene scintillation fluid.

For determination of the total incorporation of [^{3}H]uridine into acid-insoluble substances, the monolayers from which Cl_3AcOH-soluble materials were removed (see above) were dehydrated twice with 5 mL of 95% ethanol, dried, and dissolved in 4 mL of 1 M NaOH per plate at 37 °C for 20 h. Aliquots were taken from each plate, acidified slightly with 0.5 M acetic acid, and mixed with Triton X-100–xylene scintillation fluid. The counting efficiency was 25–27% with an external standard. Radioactivity from four monolayers was averaged to give an experimental value. The variation among the plates was ±4%.

Results

Time Course of Inhibition of Cellular RNA Accumulation by VS Virus at 30 and 40 °C. Effect of Infection on the Soluble [^{3}H]Uridine Pools. For the purpose of determining the time course of incorporation of [^{3}H]uridine into acid-insoluble material and into the soluble pool at 30 and at 40 °C, monolayers of L cells were infected with VS virus and pulse-labeled with [^{3}H]uridine at different times after adsorption as described under Materials and Methods. The monolayers were then extracted for acid-soluble counts and also analyzed for acid-precipitable materials. The results, shown in parts A and B of Figure 1, represent the percent incorporation per monolayer of infected cells divided by the incorporation per monolayer of uninfected cells. As can be seen in Figure 1A, the initiation of inhibition of incorporation into acid-insoluble material (presumably RNA for the most part) was faster at 40 °C than at 30 °C. Noticeable inhibition of RNA accumulation did not occur at 30 °C until ~3 h after infection but began immediately after virus adsorption at 40 °C.

Figure 1B shows the effect of virus infection on the acid-soluble pools at 30 and 40 °C. At 40 °C the incorporation of [^{3}H]uridine into the acid-soluble pool increased, in contrast to the incorporation of this isotope into acid-insoluble material, as shown in Figure 1A. The increase of labeling of the acid-soluble pool at 40 °C reached a stable level between 2 and 4 h after infection and then slowly declined. In contrast, the labeling of the acid-soluble pools in infected cells incubated at 30 °C changed less than that at 40 °C but was still higher than that in control, uninfected cells. These results suggest that the decreased incorporation of [^{3}H]uridine into acid-insoluble material does not result from a decreased content of [^{3}H]uridine in the acid-soluble pools. In fact, the amount of uridine inside the cell is increased at 40 °C.

Inhibition of Total RNA Synthesis by ts Mutants at the Permissive (30 °C) and Nonpermissive (40 °C) Temperatures. For determination of which viral gene functions are involved in the inhibition of total cellular RNA accumulation, various ts mutants of VS virus were screened for their ability to inhibit cellular RNA accumulation at 30 and at 40 °C. In these experiments L cells were infected with wild type VS virus or various VS virus mutants and labeled with [^{3}H]uridine as described under Materials and Methods and in Table I. Total RNA was extracted from whole cells by the phenol–NaDodSO$_4$ method described under Materials and Methods, and the specific radioactivity (counts per minute per microgram) of the RNA from control cells and cells infected by the different ts mutants was determined. Table I shows the percent inhibition of total cellular RNA accumulation at 30 and 40 °C at MOI values of 10 or 50.

It is clear that at 30 °C infection of L cells by wild type VS virus or the different ts mutants resulted in the inhibition of total RNA accumulation. The percent inhibition relative to the wild type varied somewhat among the ts mutants with

Table I: Percent Inhibition of Total RNA Accumulation in L Cells Infected with Wild Type VS Virus or ts Mutants at Different Multiplicities of Infection

mutant	viral protein affected	MOI	30 °C[a] % inhibn	40 °C[b] % inhibn	phenotype[c] tra	psi	ckp	rni
ts G114 (I)	L	50	22 ± 5	−1 ± 3	−	−	−	−
		10	15	4	−	−	−	−
ts G11 (I)	L	50	34	40	+	−	−	+
		10	26	30	+	−	−	+
ts G22 (II)	NS	50	20 ± 3	1 ± 3	+	−	−	−
		10	19	−1	+	−	−	−
ts O52 (II)	NS	10	22 ± 1	42 ± 2	+	nt	+	+
ts G31 (III)	M	10	27	17	+	+	+	+
		3	24	19	+	+	+	+
ts G33 (III)	M	10	31 ± 2	43 ± 3	+	+	+	+
ts G41 (IV)	N	50	30 ± 3	38 ± 4	+	−	−	+
		10	20 ± 5	28 ± 5	+	−	−	+
ts W10 (IV)	N	50	34 ± 1	55 ± 8	+	+	+	+
		10	26	38	+	+	+	+
ts O45 (V)	G	50	38 ± 6	50 ± 5	+	+	+	+
		10	25	32	+	+	+	+
ts O110 (V)	G	24	34	44	+	+	+	+
wt[d]		50	40 ± 3	59 ± 2	+	+	+	+
		10	31	43	+	+	+	+

[a] Cells were infected with VS virus and incubated for 3 h after infection at 30 °C followed by 2 h of incubation with 25 μCi/mL [^{3}H]-uridine. [b] Cells were infected with VS virus and incubated at 40 °C for 3 h after infection followed by 1 h of incubation with 25 μCi/mL [^{3}H]uridine. [c] Abbreviations: tra = primary transcription; psi = protein synthesis inhibition as per Marvaldi et al. (1977); ckp = cell killing particle activity as per Marcus & Sekellick (1975) and Marvaldi et al. (1977); rni = RNA synthesis inhibition as per this report; nt = not tested. The "+" and "−" designations denote the presence or absence, respectively, of measurable activity for each virus at 40 °C. [d] wt = wild type VS virus.

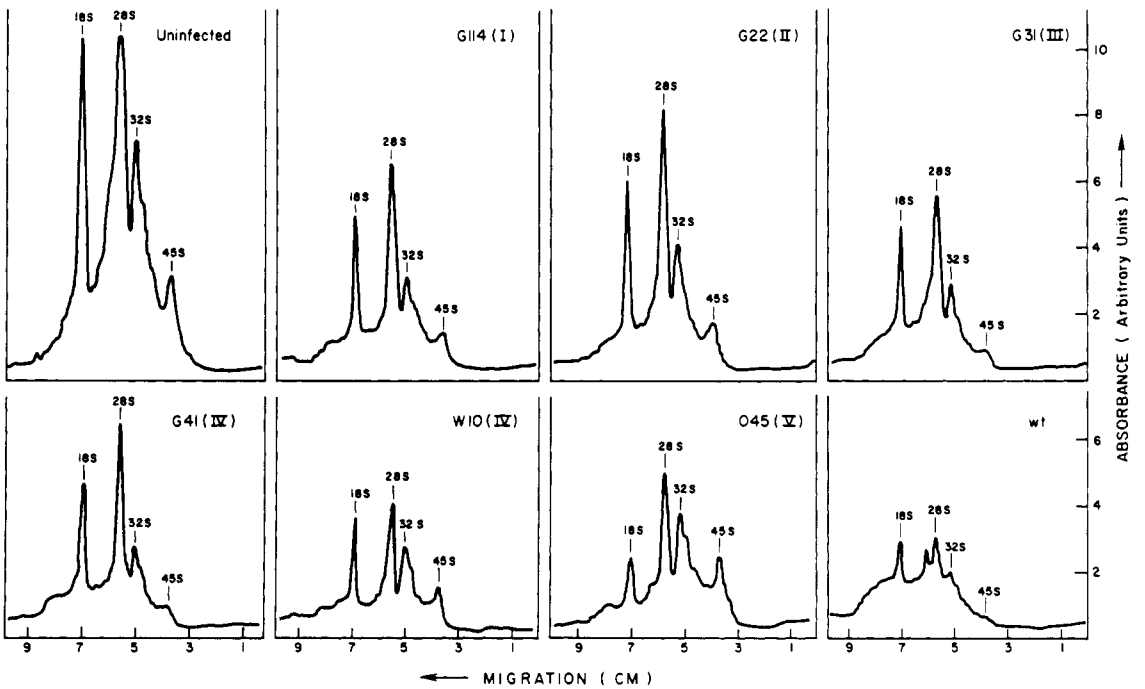

FIGURE 2: Scanning profiles of fluorogram from gel electrophoresis of RNA extracted from L cells infected with ts mutants and wild type VS virus at 30 °C. Cells were infected with VS virus at an MOI of 10, incubated for 3 h at 30 °C, and then labeled with [^{3}H]uridine for 2 h. The RNA was extracted and analyzed as described under Materials and Methods.

mutants ts G114 (I) and ts G22 (II) being ~50% less inhibitory than mutants ts W10 (IV) or ts O45 (V), for example.

At 40 °C there were large differences within the classes of mutants in their ability to inhibit cellular RNA accumulation. As expected from the results shown in Figure 1A, the percent inhibition of RNA accumulation by wild type VS virus and several of the ts mutants [ts O45 (V), W10 (IV), O52 (II), G33 (III), G41 (IV), and G11 (I)] was greater at 40 °C than at 30 °C. However, mutants ts G114 (I) and G22 (II) at 40 °C seemed to have lost the capacity to inhibit cellular RNA accumulation (compare these mutants at 30 and at 40 °C). Mutant ts G31 (III) was also able to inhibit cellular RNA accumulation at 40 °C but to a lesser degree than at 30 °C.

The inhibition of total cellular RNA accumulation in most cases was influenced somewhat by the MOI; at an MOI of 50, the inhibition was greater than that at an MOI of 10.

Inhibition of Ribosomal Ribonucleic Acid (rRNA) Accumulation by ts Mutants at 30 and 40 °C. Although much of the total cellular RNA species labeled by [^{3}H]uridine is rRNA, the effect of infection by wild type VS virus and the various ts mutants on rRNA accumulation in particular was examined at 30 and 40 °C. As shown in Figure 1A, the rate

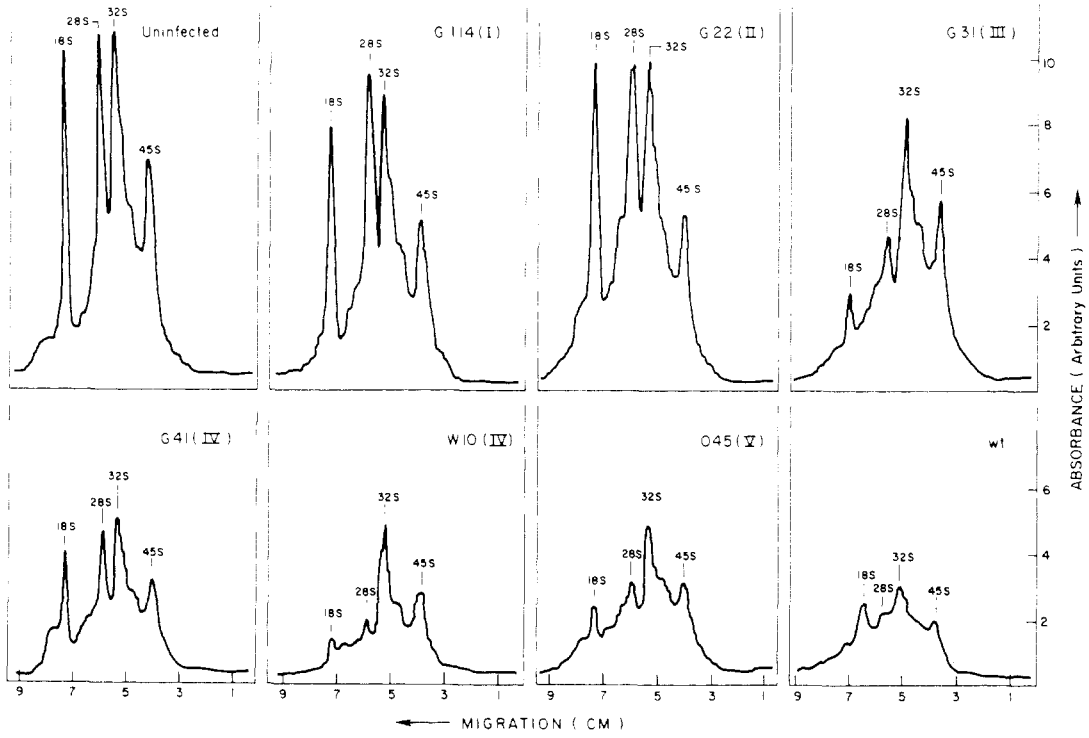

FIGURE 3: Scanning profiles of fluorogram from gel electrophoresis of rRNA extracted from L cells infected with ts mutants and wild type VS virus at 40 °C. Cells were infected with VS virus at an MOI of 10 and 4 h after infection were pulse-labeled for 1 h with 25 μCi/mL [^{3}H]uridine. The RNA was extracted and analyzed as described under Materials and Methods.

of inhibition of RNA accumulation was slow at 30 °C. The rates of rRNA synthesis and processing were also slow at this temperature. Thus, a long labeling period was used to be sure that all the rRNA precursors were labeled and processed.

Figure 2 shows the polyacrylamide gel electrophoretic pattern of rRNA labeled and extracted from infected cells as described under Materials and Methods. The first upper left panel shows the high molecular weight mature, intermediate, and precursor rRNA species accumulated in uninfected L cells at 30 °C. In each panel the same amount of RNA was applied to the gel, and thus the differences between the panels reflect differences in synthesis and/or processing of each species of RNA. The lower right panel shows the effect on rRNA accumulation of infection by wild type VS virus. The results of infection by the various ts mutants are shown in the other panels. As can be seen, there was a marked inhibition of cellular rRNA accumulation in each case. Infection by wild type virus caused the greatest extent of inhibition, followed by mutants W10 (IV), O45 (V), G31 (III), G41 (IV), G114 (I), and G22 (II). This slightly different degree of inhibition within the mutants followed the pattern of total RNA synthesis inhibition described earlier in Table I.

The synthesis of virus-specific RNA was not discernible in the gels probably because it comprised only a small fraction of the total cellular RNA labeled and isolated as described under Materials and Methods. Generally, however, the background preceding the 18S rRNA peak was higher in infected cells than in uninfected cells. This background may represent 12–18S virus-specific mRNA.

The results of infection by wild type VS virus and ts mutants at 40 °C are shown in Figure 3. In these experiments infected L cells were labeled with [^{3}H]uridine for 1 h at 4 h after adsorption and the rRNA was extracted as described above. The first upper left panel shows the rRNA species accumulated in the control, uninfected L cells, and the extreme right lower panel shows these same species labeled after L cells are infected with wild type virus. As seen in the upper second and third panels, rRNA accumulation was hardly affected in cells infected with mutants ts G114 (I) or G22 (II). Mutants W10 (IV), O45 (V), and G41 (IV) inhibited rRNA accumulation nearly as well as the wild type and to a greater extent at 40 °C than at 30 °C, as also seen in Figure 1A and Table I. Mutant G31 (III) was again less inhibitory at 40 °C than at 30 °C.

Effect of Infection by UV-Irradiated Virus on Cellular RNA Synthesis. Increasing doses of UV irradiation of virus will progressively destroy specific viral genes. Expression of each gene depends on the map location of the particular gene (Ball & White, 1976; Abraham & Banerjee, 1976) on the genome. This type of analysis gives an indication of whether newly synthesized viral proteins are necessary for expression of certain functions or whether virion proteins will suffice. L cells were infected with unirradiated VS virus or virus that had been irradiated for various times. At the time periods indicated in the legend to Figure 4, the cells were labeled with [^{3}H]uridine for 30 min and the labeled RNA was precipitated with acid as described under Materials and Methods. The inhibition of cellular RNA accumulation decreased as the exposure time of the virus to UV light was increased. The survival curves for inhibition of total RNA accumulation are shown in Figure 4. These curves represent three different experiments to point out unexplained variations seen from experiment to experiment. The target size ranged from a maximum of 17% of the genome to a minimum of 11% of the genome. For comparative purposes, the survival curve for infectivity is also shown in Figure 4. The UV dose for 37% survival of infectivity was 52.2 ergs/mm^2.

The survival curve for the N gene, which represents ~17% or one-sixth of the genome, could not be determined accurately at these times after infection, probably because of degradation of the viral protein [see also Ball (1977)].

Discussion

In this report we have attempted to determine the VS virus

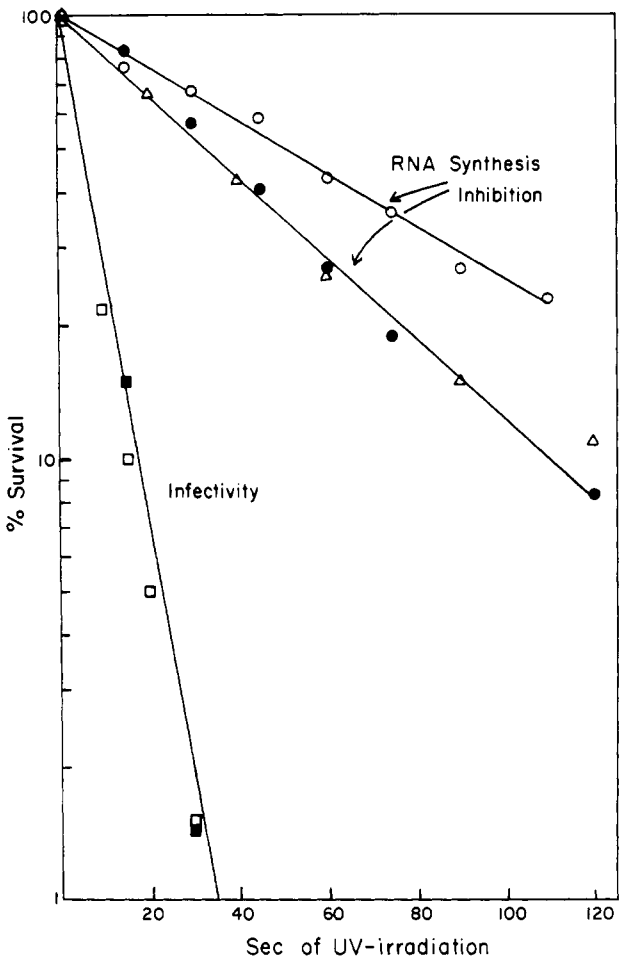

FIGURE 4: Effect of infection by UV-irradiated VS virus on expression of cellular RNA synthesis inhibition. Sucrose gradient purified wild type VS virus was UV irradiated for the periods indicated in the figure. Cells were infected with the virus at an MOI of 1 and at the times indicated below were labeled with [^{3}H]uridine for 30 min at 37 °C. For determination of infectivity, the UV-irradiated virus was titered by plaque assay: the symbols □ and ■ represent two different experiments. (△) Pulsed 5–5.5 h after infection; (○ and ●) pulsed 3–3.5 h after infection. These represent three different experiments.

genes involved in the inhibition of cellular RNA accumulation. Temperature-sensitive mutants in all five complementation groups of VS virus were screened for their ability to inhibit cellular RNA accumulation at the nonpermissive temperature to study this problem. As shown in Table I and Figure 3, the only mutants which failed to inhibit cellular RNA accumulation at the nonpermissive temperature were G114 (I) and G22 (II). Of all the mutants that we tested, only G114 (I) is supposedly unable to carry out either primary or secondary transcription at the nonpermissive temperature (Perlman & Huang, 1973; Marcus & Sekellick, 1975) due to the presence of a thermolabile transcriptase or L protein (Hunt & Wagner, 1974; Ngan et al., 1974; Marcus & Sekellick, 1975). The other group I mutant that was tested in this report, ts G11, is capable of some primary transcription in vivo at the nonpermissive temperature (M. J. Sekellick and P. I. Marcus, personal communication), has a thermostable virion (Pringle & Duncan, 1971), and is only moderately temperature sensitive in transcriptase activity in vitro (Hunt & Wagner, 1974). This mutant inhibited RNA accumulation at the nonpermissive temperature.

Primary transcription alone is not sufficient for cellular RNA synthesis inhibition, however, since mutant ts G22 (II), which most likely specifies the NS protein, is capable of some viral RNA and protein synthesis at the restrictive temperature (Wunner & Pringle, 1972; Pringle & Duncan, 1971) and yet was unable to inhibit cellular RNA synthesis at the nonpermissive temperature. In contrast to these results, ts O52, another group II mutant which is also capable of primary transcription (Flamand & Bishop, 1973), inhibited cellular RNA synthesis nearly as well as the wild type virus at the nonpermissive temperature. Our interpretation of these results is that the NS protein has multiple functions. In mutants G22 and O52, the transcriptase function is thermostable and hence primary transcription takes place in vivo. In mutant G22, but not in mutant O52, the function that is involved in the inhibition of RNA synthesis is thermolabile. Our lack of knowledge about the role of NS protein in transcription and replication does not permit further speculation about the nature of this thermolabile function of NS.

All the other mutants that were tested, including G31 (III), G33 (III), W10 (IV), G41 (IV), O45 (V), and O110 (V), were able to suppress cellular RNA accumulation at 40 °C (see Table I and Figure 3). All are able to synthesize viral mRNAs at the nonpermissive temperature but to different extents. The inhibition of RNA accumulation by mutant G31 (III), which has a defective M protein (Lafay, 1971, 1974; Pringle, 1977), was less severe at 40 °C than at 30 °C. However, the opposite results were obtained with mutant G33, also of group III. No explanation is proposed at this time for this difference in effect on cellular RNA synthesis inhibition.

Recently, McSharry & Choppin (1978) reported that isolated G protein caused an early inhibition of [^{3}H]uridine incorporation into RNA under conditions in which the transport and phosphorylation of uridine are not changed. In the results reported in this paper, neither ts O45 (V) nor O110 (V), which are defective in G protein (Deutsch & Berkaloff, 1971; Printz & Wagner, 1971; Lafay, 1974), was impaired in its ability to inhibit RNA accumulation. At this time we cannot interpret their results in light of our own other than to note that they used high concentrations of G protein.

On the basis of the mutant analyses reported in this paper (Table I), it is apparent that the requirements for the expression of cellular RNA synthesis inhibition are somewhat different from those for cellular protein synthesis inhibition and cell killing. At the restrictive temperature G11 (I) and G41 (IV) as well as G114 (I) and G22 (II) were impaired in their ability to inhibit protein synthesis and kill cells. G11 (I) and G41 (IV), however, were able to inhibit cellular RNA synthesis under these same conditions. Mutant G41 (IV) has a defective N protein (Combard et al., 1974; Freeman et al., 1978) and is capable of only limited mRNA synthesis at the restrictive temperature (Unger & Reichmann, 1973; Marcus & Sekellick, 1978). These results suggest that cellular RNA synthesis inhibition is not a consequence of cellular protein synthesis inhibition, since in two instances, G11 and G41, protein synthesis was not inhibited but RNA synthesis was.

The results of the mutant analysis suggested that viral RNA transcription was necessary for expression of RNA synthesis inhibition. Wild type VS virus was irradiated with UV light for different time periods to investigate this possibility further. If the virion proteins themselves were capable of inhibiting cellular RNA accumulation, then the inhibition would be insensitive to UV irradiation. If, on the other hand, transcription of the viral genome were necessary for the inhibition, then expression of the inhibition would be sensitive to UV irradiation of the virus. Furthermore, this method would give an indication of the size of the genome necessary for expression

of the inhibition (Ball & White, 1976; Abraham & Banerjee, 1976).

As shown in Figure 4, increasing doses of UV irradiation caused the virus to lose its ability to inhibit cellular RNA accumulation, thereby providing more evidence that transcription is necessary for expression of this phenotype in L cells. Although we cannot define the exact target size because of the variations observed from experiment to experiment, it appears to be approximately the size of the gene for N protein. The target size for expression of protein synthesis inhibition and cell killing appears to be as large as the N plus NS genes, or ~22% of the genome (Marvaldi et al., 1978).

Recently, Weck et al. (1979) have found that the capacity of VS virus to shut off cellular transcription was quite resistant to UV irradiation. We think that the difference between their results and those reported here is in the cell type used for these studies. In their case mouse myeloma cells, MPC-11, were used. There is evidence that the kinetics of inhibition of RNA synthesis is different in different cell lines (Baxt & Bablanian, 1976; Weck & Wagner, 1978).

The inhibitory effect of VS virus on cellular RNA synthesis is a gradual process and depends on the temperature. Figure 1A showed that at 40 °C [^{3}H]uridine incorporation by infected cells decreased steadily over a period of 6 h. At 30 °C there was no inhibition until 3 h after infection, and even then the inhibition was slow and gradual, being only 35% at 6 h. These results suggest that the components necessary for inhibition require a certain time period to establish an interaction with the host cell before they can inhibit cellular RNA synthesis. The delay of inhibition of host RNA synthesis caused by low multiplicities of infection (Wagner & Huang, 1966) and the inability of the virus to inhibit RNA synthesis at 4 °C (Yaoi et al., 1970) further support the above hypothesis.

In these studies the labeling of rRNA with [^{3}H]uridine represents a combination of synthesis, processing, and degradation (accumulation). It is apparent that not only is synthesis of rRNA reduced after virus infection but also the maturation of rRNA is affected. In fact, in the experiments shown in Figure 3, it appears as though the 45S pre-rRNA in infected cells continued to be transcribed and processed into intermediate species (41 and 32 S) at a reduced rate, but formation of mature (18S and 28S) rRNA is profoundly depressed.

In summary, the data presented in this report suggest that virion proteins themselves are not sufficient for inhibition of cellular RNA accumulation. The requirements for expression of cellular RNA synthesis inhibition include the following: (a) minimally functional virion L protein; (b) transcription of a part of the VS virus genome corresponding to approximately the size of the N gene (17% of the genome); (c) some as yet unknown function of NS protein which is also needed for protein synthesis inhibition and cell killing. Our results also suggest that cellular RNA synthesis inhibition is not necessarily a consequence of cellular protein synthesis inhibition.

Acknowledgments

We thank Dr. Philip Marcus for his valuable suggestions during the course of this work.

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