INHIBITION OF RIBONUCLEIC ACID METHYLATION IN THE FOOT-AND-MOUTH DISEASE VIRUS-INFECTED HOST CELL

George F. Vande Woude, Ralph B. Arlinghaus, and Jerome Polatnick

Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture
Greenport, New York 11944

Received October 9, 1967

Inhibition of baby hamster kidney (BHK) host cell protein and RNA synthesis following infection with foot-and-mouth disease virus (FMDV) has been described (Brown et al., 1966; Polatnick et al., in press). Foot-and-mouth disease virus (type A, strain 119) infection causes a more drastic inhibition of host cell protein synthesis than of RNA synthesis throughout the infectious cycle. For example, early in infection (90 min postinfection [PI]), and at peak virus production (300 min PI), protein synthesis is inhibited approximately 50 and 80%, respectively, while RNA synthesis is inhibited only 20 and 50% (Polatnick et al., in press).

In this report, the effect of FMDV infection on host cell RNA methylation is examined at 300 min PI. Methylation of total host cell RNA, fractionated 1 M NaCl insoluble RNA and sRNA is shown to be inhibited to the same degree as host cell protein synthesis in the FMDV infected cell.

Materials and Methods

Baby hamster kidney cells were grown in 2-liter Baxter bottles on roller mills (Polatnick and Bachrach, 1964). Experiments were conducted on six-day-old cells (ca. 8×10^8 cells/bottle). A multiplicity of 10 plaque forming units of FMDV per cell was used in all experiments. Virus was added to each bottle in 10 ml of minimal medium (Polatnick, 1967) and by the virus adsorption procedure previously described (Arlinghaus et al., 1966). Noninfected cells were

treated at zero time with the same medium minus virus. At 270 min PI, both non-infected and infected cells were pulse-labeled with ^3H (methyl) methionine (New England Nuclear Corporation, SA = 49.3 mC/m mole). The cells were harvested at 300 min PI.

Total RNA was extracted from noninfected and infected cells with phenol and sodium dodecylsulfate as previously described (Arlinghaus et al., 1966). Sucrose gradient analyses were performed by the method of Arlinghaus et al. (1966).

For the analysis of incorporation of ^3H methyl groups into 1 M NaCl insoluble RNA and sRNA, BHK cells were extracted with phenol, and the RNA in the aqueous phase was subfractionated with NaCl and isopropanol by the Zubay procedure (1962). The protein present in the phenol phase was precipitated overnight (-20°) by the addition of 0.1 volumes of 20% potassium acetate and 10 volumes of cold methanol. The pellet obtained by low-speed centrifugation was washed twice with 10 volumes of cold methanol, dissolved in 25 volumes of 1 N NaOH and reprecipitated with 10 volumes of 5% trichloroacetic acid (TCA). The 1 M NaCl insoluble RNA obtained from the aqueous phase was used for isotopic specific activity determination and for chromatographic analysis after alkaline hydrolysis. The sRNA was dissolved in 0.01 M sodium acetate pH 5.0 and used for isotopic specific activity determination and methylated-albumin-kieselguhr (MAK) chromatography (Sueoke and Yamane, 1962). Ribonucleic acid concentration was determined at 260 mμ using an extinction coefficient of 240. Protein concentration was determined by the biuret reaction. Radioactivity of 5% TCA insoluble ^3H (methyl) methionine labeled protein and ^3H methylated RNA was determined by liquid scintillation counting as described by Arlinghaus et al. (1966).

The 2',3'monophosphates of guanosine (G), adenosine (A), cytidine (C) and uridine (U) obtained from alkaline RNA hydrolysates (0.33 N KOH, 16 hr at 37°) were separated by thin-layer chromatography and subsequently analyzed for ^3H methyl label. Chromatography was carried out on glass plates coated with a 250 μ layer of cellulose MN 300 (Brinkmann Instruments) for 3 hr at 37° in an isopropanol: HCl:H_2O (65:16.7:18.3, by vol.) solvent system. The four nucleotides were identi-

fied by co-chromatography with standards. The UV absorbing zones were scraped from the glass plates into counting vials containing 0.5 ml of 1 N NaOH and counted as described above.

Results

Infected and noninfected BHK cells were pulse labeled at 270 min PI for 30 min with ^{3}H (methyl) methionine (100 μc/bottle). Comparison of the specific activities of noninfected and FMDV infected TCA insoluble whole cell RNA showed 80% inhibition of RNA methylation in the infected cell. Sucrose gradient analyses of these RNA preparations are shown in Fig. 1. It is apparent that the methylated RNA species found in the noninfected cell, with estimated sedimentation rates of 43, 33, 28 and 18 S, are only slightly methylated in the infected cell. The methyl label associated with the sRNA region (tubes 27-30, Fig. 1) is also considerably less in the infected cell.

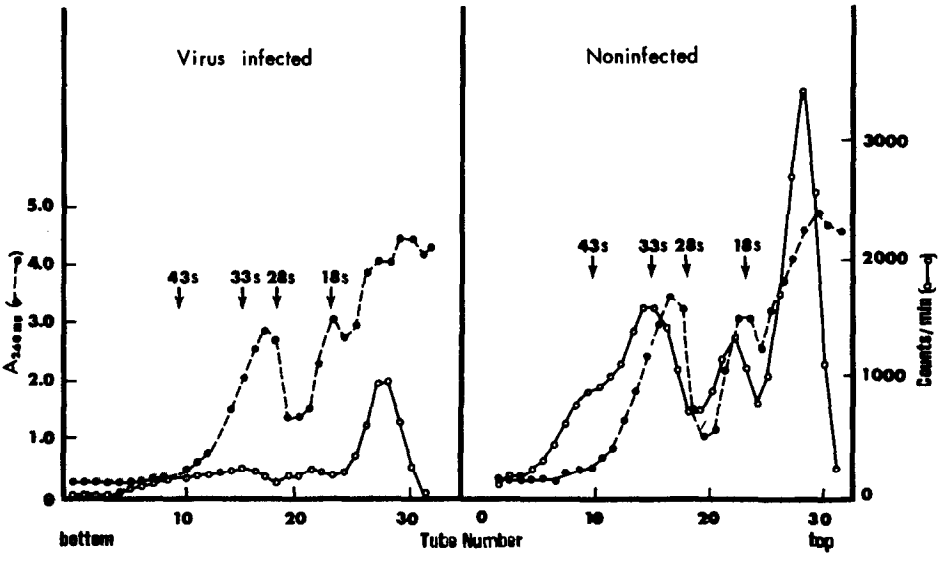

Fig. 1. Sucrose gradient analysis of total RNA extracted from noninfected and FMDV infected host cells. RNA was pulse labeled at 270 min PI with ^{3}H (methyl) methionine prior to extraction at 300 min PI. Approximately 1-2 mg of RNA was layered on (5-25% w/v) sucrose gradient, 0.01 M in sodium acetate and centrifuged for 17 hr at 24,000 rpm in a Spinco SW 25.1 rotor.

The specific activities of the protein, 1 M NaCl insoluble RNA and sRNA extracted at 300 min PI by the Zubay procedure (1962) from cells which were pulse labeled with ^3H (methyl) methionine (33 μc/bottle) at 270 min PI are shown in Table 1. The

Table 1: Protein Synthesis and RNA Methylation in Noninfected and FMDV-Infected BHK Cells

Fraction	Counts/min/mg Noninfected	Virus infected	% inhibition
1 M NaCl insoluble RNA	9,860	1,092	88.9
sRNA	84,996	15,312	82.7
Protein	15,360	1,720	88.8

methylation of the 1 M NaClinsoluble RNA fraction was inhibited as much as protein synthesis, while the sRNA fraction was inhibited 6% less.

Hydrolysates of the ^3H methyl-labeled noninfected cell 1 M NaCl insoluble RNA and sRNA and infected cell 1 M NaCl insoluble RNA (see Table 1) were examined by thin-layer chromatography (Table 2). Differences in the distribution of the

Table 2: Thin Layer Chromatography of Methylated Nucleotides Obtained from Hydrolysates of 1 M NaCl Insoluble RNA

RNA fraction	Major Nucleotides** G	A	C	U	Total Counts
1 M NaCl insoluble RNA (virus infected)*	50.6	17.5	17.5	14.5	(235)
1 M NaCl insoluble RNA (noninfected)***	47.7	15.3	22.8	14.2	(3470)

*Pulse-labeled methylated RNA was obtained as described in Materials and Methods.

** The percent of the total methyl label associated with the zone of each nucleotide. Guanylic acid included a region one-half the distance from the origin to the zone perimeter.

*** Noninfected cell sRNA was used as a control to demonstrate the resolving power of this technique. This RNA, which has a methylated base composition different from ribosomal RNA, gave percentages of ^3H methyl label of 12.4, 17.2, 63.5 and 6.8 associated with the G, A, C, U regions, respectively.

methylated bases were resolved between the two noninfected cell RNA species. No variations in the distribution of ^3H methyl label was resolved, however, in the chromatographed nucleotides of 1 M NaCl insoluble RNA from noninfected and infected cells. Thus, following virus infection there is a marked inhibition

in the methylation of all nucleic acid bases of the 1 M NaCl insoluble RNA since there was no apparent change in the percentages of ^{3}H methyl label associated with each nucleotide zone.

Fractionation of the sRNA from noninfected and infected cells by MAK chromatography is shown in Fig. 2. The sRNA extracted from infected cells showed a uniformly low level of RNA methylation throughout the chromatogram implying that the methylation of nucleic acid bases in all sRNA species is inhibited.

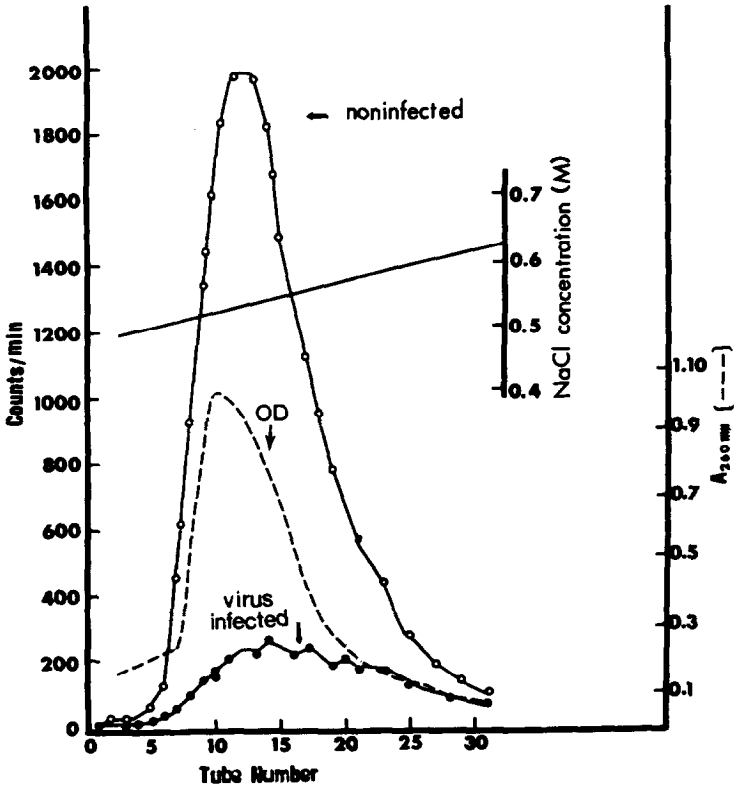

Fig. 2. Methylated-albumin-kieselguhr chromatography of ^{3}H (methyl)-labeled sRNA extracted from noninfected and FMDV-infected BHK cells. The counts have been normalized to a single A 260 mμ OD profile. Counts/min; noninfected (○); infected (●).

Discussion

At 300 min PI, host cell RNA methylation in the FMDV infected cell is inhibited to the same extent as protein synthesis. This RNA methylation inhibition parallels protein synthesis inhibition throughout the infectious cycle (Vande Woude and Polatnick, in preparation), in spite of the fact that total RNA synthesis is inhibited to a lesser extent (Polatnick et al., in press).

Several laboratories have shown that mammalian ribosomal RNA precursor acquires methyl groups before completion of the ribosome (Zimmerman and Holler, 1967; Penman et al., 1966; Saponara and Enger, 1966). Thus, inhibition of ribosomal RNA methylation by virus infection (Fig. 1, Table 1) could produce inactive ribosomes, thereby causing an inhibition of host cell protein synthesis.

In E. coli it is known that submethylation of certain RNA species results in miscoding during translation (Peterkofsky et al., 1966; Revel and Littauer, 1966). We have shown that sRNA is submethylated in the FMDV-infected cell (Fig. 2). Thus, one could postulate that FMDV associated submethylation of sRNA could alter BHK host cell translation.

The specific methylated bases of the 1 M NaCl insoluble RNA extracted from BHK cells were not identified. The chromatographic procedure did resolve differences between the methylated bases in sRNA and 1 M NaCl insoluble RNA extracted from noninfected BHK cells. No apparent differences were resolved between the methylated nucleotides of the 1 M NaCl insoluble RNA extracted from noninfected and infected BHK cells. The overall nonspecific decrease in the methylation of the nucleotides in the RNA molecule would indicate that inhibition occurs at a general level late in the infectious cycle. Perhaps an S-adenosyl methionine cleaving enzyme may be produced subsequent to FMDV infection, similar to the situation arising during T_3 phage infection of E. coli (Gefter et al., 1966).

References

Arlinghaus, R., Polatnick, J., & Vande Woude, G., Virology 30, 541 (1966).
Brown, F., Martin, S.J., & Underwood, B., Biochim. Biophys. Acta 129, 166 (1966).

Gefter, M., Hausmann, R., Gold, M., & Hurwitz, J., J. Biol. Chem. 241, 1995 (1966).
Penman, S., Smith, I., Holtzman, E., & Greenberg, H., Natl. Cancer Inst. Monograph No. 23, 489 (1966).
Peterkofsky, A., Jesensky, C., & Capra, J.D., Cold Spring Harbor Symp. Quant. Biol. 31, 515 (1966).
Polatnick, J., Appl Microbiol. 15, 228 (1967).
Polatnick, J., & Bachrach, H.L., Appl. Microbiol. 12, 368 (1964).
Polatnick, J., Vande Woude, G., & Arlinghaus, R., Archiv. ges Virusforsch. (in press).
Revel, M., & Littauer, U.Z., J. Mol. Biol. 15, 398 (1966).
Saponara, A.G., & Enger, M.D., Biochim. Biophys. Acta 119, 492 (1966).
Sueoke, N., & Yamane, T., Proc. Natl. Acad. Sci. 48, 1454 (1962).
Zimmerman, E.F., & Holler, B.W., J. Mol. Biol. 23, 149 (1967).
Zubay, G., J. Mol. Biol. 4, 347 (1962).