Inhibition of Rubella Virus-Specific RNA Synthesis by Interferon (35324)

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We have recently described the synthesis of new species of RNA in rubella virus (RV)-infected BHK-21/WI-2 cells and have suggested that the new RNA is RV RNA, although there was no direct evidence for this (14). We were not able to show infectivity for the new RNA and RNA was not successfully recovered from rubella virions for comparison with the infected cell RNA species because of the relatively poor virus yields in BHK-21/WI-2 cells. To obtain more direct evidence that the new RNA is in fact RV RNA, we have now studied RNA synthesis in RV-infected Vero cells, a stable line of African green monkey kidney cells. Vero cells do not produce interferon (IF) when infected with a number of RNA viruses (1) but replication of these viruses in Vero cells is inhibited by exogenous IF (1). This provided an opportunity to study the effects of exogenous IF on replication of RV in Vero cells. We have found that the same new RNA species are synthesized in Vero cells as previously described in BHK-21/WI-2 cells infected with RV. In addition, IF inhibits virus production and synthesis of the new RNA species in RV-infected Vero cells strongly suggesting that the new RNA is viral RNA.

Materials and Methods. Cells and virus. A continuous monkey kidney (Vero) cell line (15) was obtained from Dr. J. S. Rhim, Microbiological Associates, Bethesda, Maryland, at the 120th passage level. The media and techniques for propagation of Vero cells were the same as reported for BHK-21/WI-2 cells (14). The preparation of Gilchrist strain RV and method used to infect the cultures were identical to those reported elsewhere (13).

Interferon assay. Monkey IF, prepared as previously described (13), was titrated in Vero cells with vesicular stomatitis virus as the challenge virus. This pool contained about 300 units/ml and had all of the generally described characteristics of IF (13).

Plaque assay. The virus was assayed as described by Rhim and Schell (11).

Reagents. Actinomycin D (AD) was purchased from Merck, Sharp, and Dohme, Rahway, N.Y. ^{3}H-Uridine (25 Ci/mmole) was purchased from Schwarz BioResearch Inc., Orangeburg, N.Y., and from New Enggland Nuclear Corp., Boston, Mass.

RNA extraction and sucrose density gradient analysis. Methods for RNA extraction, sucrose density gradient analysis and the assay for acid-precipitable radioactivity in sucrose gradient fractions were conducted as previously described (14).

Results. Inhibition of RV-specific RNA synthesis. A high virus titer was obtained in RV-infected Vero cells (6). RV used in this study grew to the same virus titers as in RV-infected BHK-21/WI-2 cells but the pattern of virus growth was different. In Vero cells, the infectious virus began to increase 24 hr after infection and continued exponentially until 96 hr after virus infection. The virus yields ($10^{7.0}$ PFU/ml) from RV-infected Vero cultures were employed as a virus seed for these experiments.

The synthesis of virus-specific RNA was observed at various times after RV infection by incubating cell cultures with ^{3}H-uridine 2 hr after addition of actinomycin D (AD). AD at 100 μg/ml inhibited ^{3}H-uridine incorporation into the RNA of uninfected cells by more than 99%. ^{3}H-Uridine incorporation

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TABLE I. Reduction of Rubella Virus-Specific RNA Synthesis in Interferon-Treated and Virus-Infected Vero Monkey Kidney Cell Cultures.

Treatment (hr)	Total TCA-precipitable radioactivity (cpm)[a]	Virus titer (log_{10} PFU/ml)	Reduction of cpm (%)	Inhibition of virus (%)
Infected cells:				
Interferon for 21	77,120	4.2	73.00	96.0
2	244,260	5.7	20.00	0.0
Trypsin-treated interferon for 21	292,990	5.6	0.00	0.0
None	292,600	5.6	0.00	0.0
Uninfected cells:				
Interferon for 21	50,300	0.0	—	—
None	49,480	0.0	—	—

[a] Counts per minute (cpm) for ^{3}H-uridine incorporation into RNA. Interferon used in this experiment was 50 units/ml. Actinomycin D (100 μg/ml) was added at 21 hr PI and ^{3}H-uridine (25 μCi/ml) was then added and incorporation into RNA followed. This dosage of actinomycin was required as discussed elsewhere (15) to get good inhibition of DNA dependent RNA synthesis in this cell line. It did not block RV synthesis or cause cytopathic effects in control uninfected cells in the time required for this study. Total RNA was extracted by the phenol-SDS method as previously described (14).

into the RNA of cells 16 hr postinfection was found to be 8-fold greater than in uninfected cells and 10-fold greater at 24 hr after infection. Viral-specific RNA synthesis decreased after 24 hr. In contrast, in BHK-21/WI-2 cells, viral specific RNA is first detected at 20 hr after infection and the rate increases and reaches a maximum at about 50 hr after infection (14).

The experiment in Table I was done to determine whether the synthesis of viral-specific RNA in RV-infected Vero cells was sensitive to the action of IF. Four separate Vero cultures were infected at an input multiplicity of 5–10 PFU/cell after treatment of cells with: (i) medium for 21 hr, (ii) IF (50 units/ml) for 21 hr, (iii) IF (50 units/ml) for 2 hr, and (iv) trypsin-treated IF for 21 hr. After virus adsorption for 3 hr at 35°, the virus inoculum was removed and the maintenance medium was added. Twenty-one hr after virus infection, AD was added to the cultures and 2 hr later ^{3}H-uridine (25 μCi/ml) was added. After 1 hr more of incubation, the RNA was extracted as described previously (14). The total radioactivity (cpm) from the cultures virus-specific RNA of both IF-treated and control cultures is shown in Table I. It can be seen that about five times more radioactivity (292,600 cpm) was present in RV-infected cells compared with the uninfected cells (49,480 cpm). The incorporation into the RV-infected control cells resembled that in cells which were treated with trypsin-treated IF. In the culture treated with IF for 21 hr there was a marked inhibition of virus yield (96%) and ^{3}H-uridine incorporation into RNA (74%).

Sucrose density gradient sedimentation of rubella virus-specific RNA from interferon-treated VERO cells. Figure 1C shows that ^{3}H-uridine was incorporated into 2 major RNA components designated 41S (peak around fraction 8) and 19S (peak around fraction 26) in the RV-infected culture not treated with IF. The sedimentation coefficients (S) value were calculated by the method of Martin and Ames (7). Incubation of RNA from each fraction with ribonuclease in buffer containing 0.2 *M* NaCl before TCA-precipitation revealed that the 41S RNA was rendered completely TCA-soluble and that the 19S RNA was almost completely resistant to ribonuclease digestion suggesting that it is highly base paired and possibly double stranded (3). Figure 1D shows the RNA from the culture treated with IF for 21 hr before infection and it is clear that IF has

inhibited the synthesis of both the 19 and 41S RNA species.

Figure 1A shows that treatment of the culture for only 2 hr before infection inhibited synthesis of both viral-specific RNA species by only a small amount. The percentage reduction in ^{3}H-uridine incorporation into the 19 and 41S peaks was 15 and 32%, respectively, with 2-hr treatment and with 21-hr treatment it was 80% in 19S and 84% in

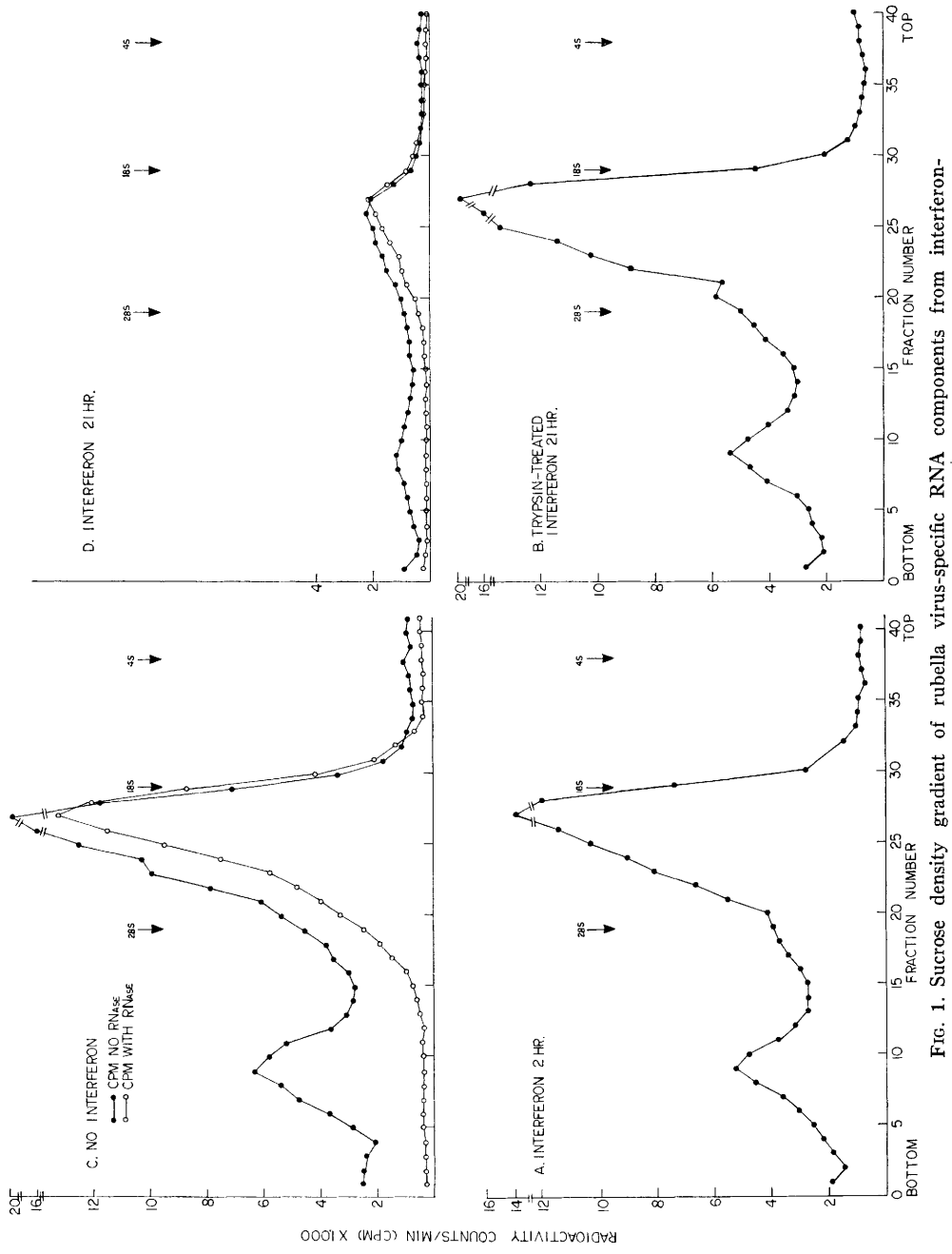

FIG. 1. Sucrose density gradient of rubella virus-specific RNA components from interferon-treated Vero cells.

41S. Figure 1B shows that trypsin-treated IF does not inhibit synthesis of either viral-specific RNA species.

Discussion. We have recently reported the synthesis of four virus-specific RNA species in BHK-21/WI-2 cells after infection with RV (14). Similar observations on the synthesis of virus-specific RNA of RV have been reported by others (5, 12). Two components of labeled RNA with sedimentation coefficients of 20–23S were found. The 38S RNA appeared to be single stranded and was thought to represent viral RNA. The 38S RV RNA was reported to be infectious (12). The ribonuclease-resistant species appeared to be double-stranded RNA and was considered to be analogous to the replicative form (12).

In this report, ^{3}H-uridine incorporation into virus-specific RNA observed in RV-infected Vero cells was studied using 100 μg/ml concentration of AD. About 5-fold higher concentration of AD was required for Vero cells than for BHK-21/WI-2 cells (20 μg/ml) (14) to inhibit host RNA synthesis by 99% and demonstrate viral-specific RNA synthesis. The same RNA species were detected in the two cell types. Adequate interferon dosages were employed in these studies to show viral inhibitory effects despite actinomycin treatment of infected monolayers just prior to radioactive uridine pulsing and RV-specific RNA isolation.

Interferon is produced by a variety of RV-infected tissue culture cells (2, 9, 10, 13). Parkman *et al.* (10) demonstrated that large amounts of IF were produced in rubella-infected GMK cells by a vaccine strain attenuated for man. The sensitivity of RV to monkey IF was studied by Wong *et al.* (13), who demonstrated that rubella virus was more sensitive to IF than vesicular stomatitis virus in monkey cells. There is evidence that RV may manifest different sensitivity to IF in different species of host cells (2).

In this study we show that in Vero cells treated for 21 hr with IF there was a marked inhibition of viral-specific RNA synthesis. These results are similar to the observations of Gordon *et al.* (4) on the effects of IF on Mengo viral RNA synthesis. Mecs *et al.* (8) observed that synthesis of 20S ribonuclease-resistant RNA was relatively more resistant to IF action than the faster sedimenting single-stranded viral RNA component in cells treated for a short time with IF. Our data (Figs. 1A, C) suggests a similar differential effect for RV.

Summary. The effect of interferon (IF) on rubella virus (RV) replication in Vero tissue culture cells was investigated. Viral-specific RNA components were synthesized in RV-infected Vero cells in the presence of actinomycin D. The synthesis of these RNA species was shown to be inhibited by IF.

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