Ribonuclease Activity Is Associated with Subviral Particles Isolated from Interferon-Treated Vesicular Stomatitis Virus-Infected Cells

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Previously we have shown that inhibition of replication of vesicular stomatitis virus in interferon-treated JLSV-11 cells is at least partly caused by impaired viral primary transcription. Here we report that subviral particles isolated from interferon-treated infected cells were deficient in mRNA synthesis in vitro compared with the particles isolated from untreated cells. This was due to the presence of an associated ribonuclease activity which hydrolyzed not only newly synthesized viral mRNAs but also exogenously added viral transcripts.

The detailed mechanism by which interferon (IFN) inhibits the replication of a virus remains unclear despite extensive studies on the antiviral actions of IFN (6, 9). We have been studying the underlying mechanisms by which IFN inhibits vesicular stomatitis virus (VSV) replication. Using a VSV temperature-sensitive mutant and two pairs of IFN-sensitive and -resistant cell lines, we demonstrated that accumulation of VSV primary transcripts is inhibited by IFN-treatment of GM2767 and JLSV-11, two sensitive cell lines (2). Since virus entry and uncoating are not affected under these conditions, it appears that viral primary transcription itself is impaired in IFN-treated cells. Here we report that VSV subviral particles isolated from IFN-treated JLSV-11 cells, which had been restricted to primary transcription conditions, synthesized less VSV mRNA in vitro than did similar particles isolated from untreated cells. Results are presented which suggest that this was caused by the presence of a ribonuclease activity associated with the subviral particles isolated from IFN-treated cells.

We have previously described procedures for culturing JLSV-11 cells and infecting them with VSV (2), methods for VSV mRNA transcription in vitro, and analyses of the products (4) and methods for the preparation of mRNA of the phosphoprotein (NS) of VSV in vitro by using a plasmid vector containing NS cDNA inserted behind a bacteriophage SP6 transcriptional promoter (3). IFN treatment was done for 18 h with 500 U of a partially purified mouse beta IFN preparation per ml (specific activity, 2 × 10^6 U of protein per mg) purchased from Lee BioMolecular, San Diego, Calif. VSV subviral particles (RNPs) were isolated from IFN-treated or untreated infected JLSV-11 cells (10). For this purpose, cells were grown in roller bottles; at 50% confluency the cells were treated with 500 U of mouse IFN per ml for 18 h, if desired, and infected with VSV at a multiplicity of infection of 10 in the presence of 100 μM anisomycin, as described before (2). After 1 h of virus adsorption and an additional 2.5 h of virus infection in the presence of anisomycin, cells were harvested by scraping, after washing them three times in cold phosphate-buffered saline. Cell pellets were suspended in a cold buffer containing 10 mM Tris hydrochloride (pH 7.5)—1.5 mM MgCl2—10 mM KCl—1 mM dithiothreitol, left on ice for 30 min, and then homogenized in a Dounce homogenizer with 60 strokes. KCl was added to a final concentration of 150 mM, and the extract was centrifuged at 16,000 × g for 20 min. To the supernatant, 2 mM EDTA was added to dissociate the polysomes, and the mixture was centrifuged in a Ti75 rotor at 40,000 rpm for 2 h. The resulting pellet was suspended in TE buffer (10 mM Tris chloride [pH 7.5], 1 mM EDTA). Then, 150 μl of TE buffer was used to suspend the pellet obtained from 2 × 10^6 cells from one roller bottle. The protein concentration of this suspension, which was used as the source of the RNPs, was about 3.5 mg/ml.

Since we sought to reproduce in vitro the observed inhibition of VSV primary transcription in vivo in IFN-treated cells, we first examined the transcriptional ability of VSV RNPs isolated from cells that had been infected with virus in the absence of ongoing protein synthesis. The electrophoretic migration patterns and the relative quantities of VSV transcripts synthesized in vitro were very similar irrespective of whether detergent-disrupted virions (Fig. 1, lane 1) or viral RNPs isolated from BHK cells with (lane 2) or without (lane 3) ongoing protein synthesis were used. N, NS, and M mRNAs were the predominant species, whereas G mRNA was less abundant, with little L mRNA being synthesized. A similar pattern of synthesis was observed when we used VSV RNP isolated from infected JLSV-11 cells that had been treated with anisomycin (lane 4). The qualitative pattern of synthesis of different VSV mRNAs did not change appreciably even when the JLSV-11 cells were pretreated with IFN. However, the relative amount of RNA synthesis was reduced appreciably (lane 5). The extent of this inhibition varied from lot to lot of RNP preparations, which will be apparent from the other experiments described here. To guard against this variability, pairs of RNPs from IFN-treated (RNPp) and untreated (RNPc) cells were always prepared at the same time and a different pair of RNPc and RNPp was used in each experiment described here.

To quantitate the extent of inhibition more rigorously, the experiment in Fig. 2 was done with one pair of RNPc and RNPp cells. In vitro transcription was carried out with various concentrations of RNPc and RNPp, the products were analyzed by gel electrophoresis, and the amount of N mRNA synthesized in each reaction was quantitated by excising the N mRNA band from the gel and counting it in a scintillation counter. At every concentration tested, less N

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The bands representing mRNA were excised from the gel, and the associated radioactivities were quantitated by being counted in a scintillation counter. RNPC and RNPI were isolated from JLSV-11 cells infected in the presence of anisomycin.

FIG. 3. Presence of an inhibitor of transcription in RNPI. Transcription reactions were carried out for 1 h. Lanes: 1, 0.35 mg of RNPC per ml of reaction mixture; 2, 0.35 mg of RNPC and 0.35 mg of RNPI per ml; 3, 0.35 mg of RNPI per ml; lane 4, 0.70 mg of RNPI per ml. (B) Pulse-chase reactions. Standard transcription reactions with 0.35 mg of RNPs per ml were carried out for 1 h. One set of aliquots was saved for analyses; to another set of equal aliquots 20-fold excess of unlabeled CTP was added, and the incubation reactions were continued for another 1 h. Lanes: 5, RNPI transcription products before chase; 6, RNPI transcription products after chase; 7, RNPC transcription products before chase; 8, RNPC transcription products after chase. mRNA species are shown.
associated with similar pellets obtained from IFN-treated uninfected cells, suggesting that the presence of virion RNP was necessary for sedimentation of this activity (data not shown).

In the experiments described here, we have demonstrated that VSV RNPs isolated from IFN-treated JLSV-11 cells synthesized significantly less VSV mRNA in vitro than did RNPs isolated from the same number of untreated cells. This result is consistent with earlier observations of impaired VSV primary transcription in vivo (2, 8). Moreover, our data clearly indicated that this reduced RNA synthesis was caused by the action of an RNase activity associated with RNP. Purification and further characterization of the RNase activity are needed for understanding the basis of its association with VSP RNP. It remains to be seen whether this activity is related to the IFN-inducible RNase L activity which has been implicated to be responsible for impaired mRNA transcription by reoviral cores isolated from IFN-treated cells (5, 7) and by reovirions to which extract of IFN-treated cells has been added (1). Wallach and Revel (11) have reported trapping 2',5'-oligoadenylate synthetase in VSV and murine leukemia virus particles released from IFN-treated cells (11). Activation of the RNase activity associated with RNP by 2',5'-oligoadenylate synthesis in situ is however unlikely, since this RNase could hydrolyze mRNA in the absence of any added ATP. Irrespective of whether this RNase activity is related to RNase L activity, the important mechanistic question is whether it can account for the inhibition of VSV primary transcription observed in IFN-treated cells in vivo. Experiments to clarify these issues are in progress.

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