EFFECT OF INTERFERON ON SYNTHESIS OF ssRNA IN REOVIRUS TYPE 3-INFECTED L CELL CULTURES

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Summary: Interferon treatment of L cell cultures prior to infection with reovirus type 3 resulted in a dose-dependent reduction in synthesis of infectious virus and single-stranded ribonucleic acids (ssRNA). New species of ssRNA were synthesized in interferon-treated infected cell cultures, including ssRNA species larger and smaller than those normally found in infected cells.

Infection of L cells with reovirus type 3 results in synthesis of single-stranded ribonucleic acid molecules (ssRNA). Several facts suggest that the ssRNA molecules serve as reovirus messenger RNA. ssRNA have been released from polyribosomes extracted from reovirus-infected cells (1,2). ssRNA molecules extracted from infected cells hybridized exclusively with denatured double-stranded geonomic RNA (dsRNA) molecules of comparable length (3,4,5). Reovirus ssRNA molecules were synthesized in vitro using purified reovirions which contain an RNA polymerase tha transcribes each of the genomic dsRNA segments into ten unique ssRNA species of corresponding length (6). Recent evidence (7,8) suggests that ssRNA also serves a precursor molecules in the synthesis of genomic dsRNA. ssRNA plays a prominent ro in the replication of reovirus and therefore studies of the effect of interferon o the synthesis of ssRNA should be useful in understanding the mechanism of action o interferon. To this end, the present study was initiated.

MATERIALS AND METHODS

L cells were grown in suspension culture as previously described (9). Preparation of stock virus and plaque assay of reovirus type 3, Dearing strain, was previously described (3). Interferon was prepared in L cells by use of MM virus (10), and concentrated by use of $(NH_4)_2SO_4$ (11). The interferon fluids were

centrifuged at 40,000 x g for 1 hr at 4°, exposed to pH 2 for 6 hr followed by irridiation with a GE germicidal lamp (11). The interferon was assayed using vesicular stomatitis virus: one unit was that dilution of interferon which reduced formation of plaques by 50%. Three units of the international mouse reference interferon assayed as one unit under these conditions. Cell cultures were incubated with interferon for 16 to 18 hr prior to infection. Procedures for infection of L cells in suspension culture have been described (2). Zero time is the instant of suspension of infected cell cultures in Minimal Essential Medium (Joklik's modified formula) containing 1% heat-inactivated fetal calf serum, lysine (12) and 0.5 μg/ml of actinomycin D (Act D) (virus growth medium, VGM). The method for measuring the kinetics of synthesis of reovirus ssRNA and dsRNA in control and interferon-treated L cell cultures was described previously (2). dsRNA is defined as RNA that is not hydrolized to acid-insoluble material following exposure to 10 μg/ml of pancreatic ribonulcease for 30 min at 36°. Tritium-labeled ssRNA was obtained without labeling of dsRNA by treating infected cells with Act D (2 μg/ml) and cycloheximide (20 μg/ml) for 45 min at 7 hr pi followed by exposure to 1 μCi/ml of uridine-5-^{3}H for 2.25 hr (9, 13). ssRNA was extracted from cytoplasm with phenol and sodium dodecyl sulfate (SDS) (9), heated for 2 min at 70°, and centrifuged into 5 to 20% sucrose gradients containing 0.5% SDS (3). Fractions were collected onto filters and trichloroacetic acid-insoluble counts were determined (3).

RESULTS

Effect of various concentrations of interferon on yields of reovirus type 3 in L cell cultures. Cell cultures were exposed to interferon for 16 to 18 hr prior to infection. The infected cultures were harvested at 24 hr pi and virus yields were measured by the plaque technique. Two experiments which show the range of inhibition of various concentrations of interferon on virus yields are shown in Table 1. Increasing the concentration of interferon resulted in greater inhibitions of yields of virus but the level of inhibition at a given concentration of interferon varied in different experiments.

Effect of interferon on the kinetics of synthesis of viral ssRNA and dsRNA.

TABLE 1. EFFECT OF INTERFERON ON YIELDS OF REOVIRUS TYPE 3 IN L CELL CULTURES

Interferon Concentration (units/ml)	Virus Yields (%C)* Expt. 1	Expt. 2
500	0.3	-
100	0.3	13.4
20	1.1	19.0
4	3.9	36.5
0	100	100

*%C = Virus yields per cell were 750 and 1300 PFU per cell in control cultures in Expt 1 and 2, respectively.

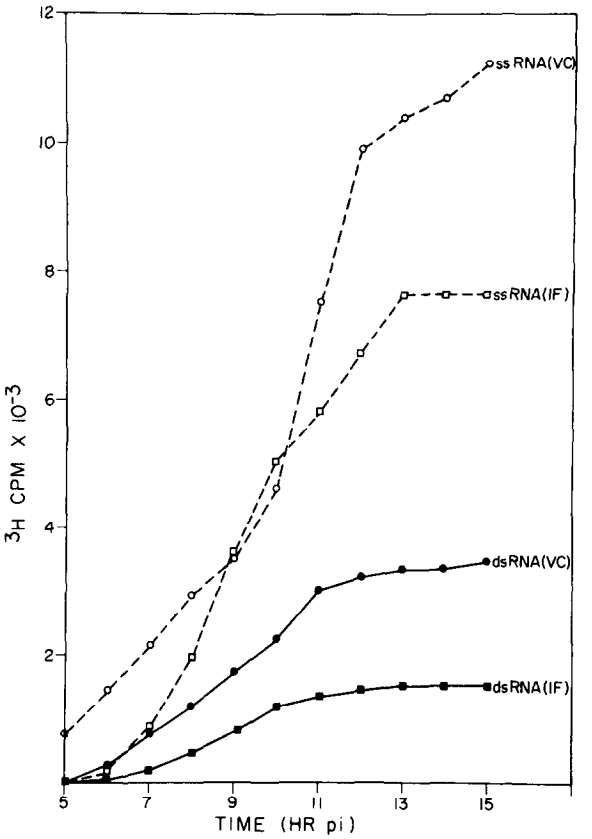

Fig. 1. Time course of synthesis of reovirus ssRNA and dsRNA in interferon-treated and control cell cultures. Cell cultures were exposed to 10 or zero units of interferon for 18 hr prior to infection. The methods for labeling, extracting and identifying ssRNA and dsRNA are described in Materials and Methods. Each point in a curve represents the cummulative total of counts in that class of RNA up to that time (hr pi). All values were corrected for incorporation of ^{3}H-counts into RNA of that class in uninfected cells. Virus yield from cells in the interferon-treated culture was 23% of the control cell culture yield of 650 PFU/cell.

The rates of synthesis of both ssRNA and dsRNA in control and interferon-treated cell cultures were similar until 10 hr pi, as shown in Fig 1. After 10 hr pi, the rates of synthesis of both classes of viral RNA were reduced in interferon-treated cell cultures. Cells in the interferon-treated culture ceased to synthesize both ssRNA and dsRNA by 13 hr pi. Interferon reduced synthesis of dsRNA and ssRNA by 57 and 32%, respectively.

Effect of various concentrations of interferon on the amount of ssRNA synthesized in infected L cell cultures. Cell cultures were treated with interferon and ^{3}H-labeled ssRNA was prepared as described in Materials and Methods. A dose-dependent inhibition of synthesis of ssRNA by interferon was observed (Table 2). Increasing the concentration of interferon resulted in an increasingly greater inhibition of synthesis of ssRNA. These experiments were selected to show the range in inhibition of synthesis of ssRNA for comparable concentrations of interfero

TABLE 2. EFFECT OF INTERFERON ON SYNTHESIS OF ssRNA IN L CELL CULTURES INFECTED WITH REOVIRUS TYPE 3*

Interferon Concentration (units/ml)		Acid-insoluble ^{3}H-counts (CPM) in ssRNA+	%C
Expt. A	40	609	33
	4	1072	57
	0	1872	100
Expt. B	50	4980	74
	5	6225	93
	0	6712	100

* L cell cultures were incubated with interferon at the indicated concentration for 16 to 17 hr prior to infection. ssRNA was labeled with ^{3}H-uridine in the presence of Act D and cycloheximide. Virus yields were 39, 952, and 1286 PFU/cell from cell cultures treated with 40, 4 or 0 units of interferon and 315, 347, and 567 PFU/cell from cell cultures treated with 50, 5 or 0 units/ml of interferon.

\+ The counts are in phenol-SDS-extracted ssRNA. Greater than 95% of the ^{3}H-counts of each sample were hydrolzed to trichloroacetic acid-soluble material by ribonuclease.

Since the same batch of interferon and same stock virus was used in these two experiments, the disparity in levels of inhibition of ssRNA synthesis by comparable doses of interferon suggest that differences in the physiological state of the cel at the time of infection plays a role in determination of virus yields.

Velocity sedimentation analysis of reovirus ssRNA synthesized in interferon-treated L cell cultures. Centrifugation of reovirus type 3 ssRNA molecules in

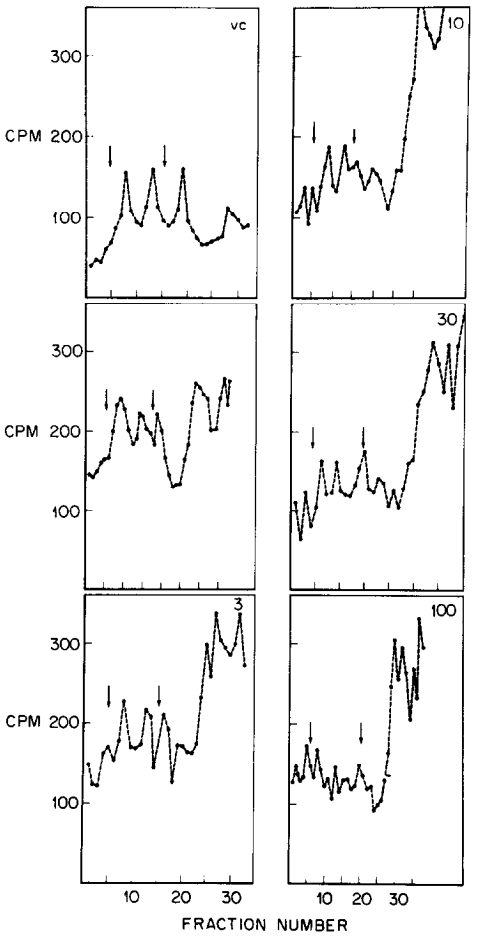

Fig. 2. Centrifugation of ssRNA extracted from interferon-treated reovirus-infected L cell cultures in sucrose gradients. Suspension cultures of L cells were exposed to various concentrations of interferon (units/ml are denoted by the number in the upper right corner of each Fig) for 18 hr. Techniques for labeling, extracting and centrifuging ssRNA onto 5 to 20% sucrose gradients are described in Materials and Methods. Virus yields in cultures exposed to 1, 3, 10, 30 and 100 units/ml of interferon were 71, 23, 12, 9 and 5% of the control yield of 250 PFU/cell. The bottom of the gradient is to the left in all Fig. The arrows represent positions to which 28 and 18S KB cell ribosomal RNA sediment

linear 5 to 20% sucrose gradients allows the resolution of three different size-classes of ssRNA molecules of average sedimentation coefficients of 24.5, 18.5 and 14.0S (3,6, 9). Similar velocity sedimentation analyses were carried out on ^{3}H-uridine-labeled ssRNA extracted from reovirus-infected interferon-treated and control L cell cultures. Greater than 95% of ssRNA analyzed in these experiments was susceptible to digestion by pancreatic ribonuclease (10 μg/ml) at 36^{o} after 30 min incubation. Under conditions that were used for labeling ssRNA in these experiments, ssRNA species with sedimentation coefficients greater than 4 to 6S were not detected by sucrose gradient analysis of RNA extracted from uninfected control or interferon-treated (50 units/ml) cell cultures. The results of sucrose gradient analyses of ssRNA species synthesized in L cell cultures exposed to different concentrations of interferon are shown in Fig 2. Approximately equal amounts of ssRNA, as judged by ^{3}H-counts in phenol-SDS-extracted acid-insoluble materials, were applied to each gradient. The results of several similar experiments are summarized in Table 3. Several general conclusions were drawn. (1) In L cell cultures exposed to most concentrations of interferon,

TABLE 3. EFFECT OF INTERFERON ON NUMBER OF SIZE CLASSES OF ssRNA SYNTHESIZED IN REOVIRUS-INFECTED L CELLS*

Number of ssRNA size-classes	Concentrations of interferon (units/ml)	Virus Yield (%C) in Expt. No. 1	2	3	4	5
3	0	100	100	100	100	100
4	1-5	71	87	94	74	76
5	3-10	23	-	15	-	-
6	8-40	12, 9	44	-	-	54
7	40	-	-	-	3	-
8	100	5	-	-	-	-

*Number of size-classes of ssRNA was determined from the RNA profiles of ssRNA centrifuged into linear 5 to 20% sucrose gradients under the conditions described in the legend for Fig 2. The data shown for Expt. No. 1 was taken from the RNA profiles shown in Fig No. 2.

the three normal size-classes of reovirus ssRNA were detected, although the amounts of ssRNA in each class were similarly reduced as the dose of interferon was increased. (2) Aberrant size classes of ssRNA were synthesized in interferon-treated cell cultures and the number and amount of these aberrant size-classes increased with increasing concentrations of interferon. The number of aberrant siz classes of ssRNA synthesized per culture showed an inverse correlation with the titer of infectious virus produced per cell culture, i.e. as yields of virus were reduced by increasing doses of interferon, an increase was found in numbers of aberrant size-classes of ssRNA. (3) Some of the aberrant size-classes of ssRNA synthesized in interferon-treated cell cultures were larger than the largest size-class of ssRNA synthesized in infected control cell cultures. (4) As the dose of interferon was increased, the infected cell cultures synthesized increased amounts of low molecular weight TCA-insoluble RNA. (5) ssRNA extracted from cell cultures which had been exposed to interferon doses that resulted in a 95% or greater inhibition of virus yields gave variable ssRNA patterns in sucrose gradients

DISCUSSION

Treatment of L cells with interferon prior to infection with reovirus type 3 reduced synthesis of normal viral ssRNA species and permitted synthesis of new species of ssRNA. Cell cultures treated with homologous interferon were reported to synthesize markedly reduced amounts of early viral messenger RNA species in cells infected with SV_{40} (15), VSV (16) and vaccinia viruses (17). The studies with VSV (16) and vaccinia (17) showed that the antiviral activity of interferon was directed against the virion-bound transcriptases. The results presented herein showed that interferon treatment of L cells prior to infection resulted in synthesis of ssRNA molecules that were larger than the largest ssRNA species found in untreate infected cells. Hybridization experiments are in progress to check the assumption that the large ssRNA molecules are viral. The existance of large ssRNA molecules implies that there must be large dsRNA templates. Large strands of reovirus dsRNA in lengths up to 5μ (18) and 7.7μ (19) have been seen in electron microscopic studies of dsRNA released from gently disrupted virions. Therefore, dsRNA segments

are most probably linked in some manner prior to incorporation into virions and could serve as templates for transcription of large ssRNA molecules. Data suggesting that large ssRNA molecules may be present in reovirus-infected L cells has been reported (2). Ward, et al. (2) found that viral polyribosomes containing more than 30 ribosomes contained equal quantities of the three size-classes of ssRNA molecules. Since the smallest size-class (14.0S) of ssRNA should accomodate 12 ribosomes at maximum, linkage of 14.0S ssRNA molecules to 24.5 or 18.0S ssRNA molecules could account for the presence of the 14.0S ssRNA molecules in the large polyribosomes (2). As large ssRNA molecules are normally not extracted from reovirus-infected cells with phenol and SDS, the results presented herein suggest that interferon alters the normal process of termination of transcription of ssRNA. Interferon treatment also resulted in an increase in synthesis of small (4 to 6S) ssRNA molecules which may represent incomplete portions of viral ssRNA molecules. If the hypothesis that ssRNA molecules serve as precursors in the synthesis of genomic dsRNA segments is correct, the results of this report indicate that interferon inhibits replication of reovirus by interfering with synthesis of precursor ssRNA molecules.

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