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Formation and characteristics of reovirus subviral particles in interferon-treated mouse L cells.

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ABSTRACT

We examined the early phase of reovirus replication in interferon-treated L cells. No difference was detected in the adsorption of virus to cells, the rate and extent of conversion of parental virions to subviral particles (SVPs), and the protein and double-stranded RNA composition of the SVPs when comparing reovirus infection of interferon-treated and control cells. Furthermore, when tested *in vitro*, SVPs isolated from interferon-treated cells (SVP_{INT}) synthesized and methylated reo mRNAs at the same rate as SVPs isolated from control cells (SVP_{CON}). However, the accumulated products of RNA synthesis promoted by SVP_{CON} consisted mainly of full size reo mRNA molecules, whereas those whose synthesis was promoted by SVP_{INT} consisted mainly of shorter products. These results indicate that premature termination of transcription and/or degradation of full size transcripts occurred *in vitro* with SVP_{INT}. Other experiments revealed that a nuclease is associated with our SVP_{INT} preparation.

INTRODUCTION

Interferons are glycoproteins whose synthesis is induced in a variety of vertebrate cells upon viral infection. They are released from the producing cells, interact with other cells and make these inefficient in supporting the growth of a broad range of viruses[1]. We have been investigating the nature of the inhibition of reovirus replication in interferon-treated mouse L cells.

Previous reports were devoted to the effect of interferon on the late phase of reovirus replication[2,3,4]. These revealed an inhibition of both viral protein and RNA (single and double-stranded) accumulation. Furthermore, preliminary results from our work[4] and that of Wiebe and Joklik[5] indicated that the early phase of reovirus replication (i.e. adsorption to cells and conversion of parental virions to subviral particles) was not altered in interferon-treated cells. These observations were recently reported in detail by Wiebe and Joklik[6]. In this communication we provide data on which our earlier conclusions were based and, in addition, provide evidence for a difference in functional characteristics, when tested *in*

in vitro, between subviral particle (SVP) preparations isolated from interferon-treated cells and control cells.

Insight into the early phase of reovirus replication in L cells was provided by the studies of Levin *et al.*[7], Chang and Zweerink[8] and Silverstein *et al.*[9]. These investigators established that after penetration into the cell the reovirions are converted into SVPs by removal and cleavage of their outer coat proteins. Each reovirion and each SVP contains ten different genomic double-stranded RNA segments. The commonly recognized capsid polypeptides of reovirions include the species λ_1, λ_2, μ_1, μ_2, σ_1, σ_2 and σ_3 whereas those of SVPs were reported to include only λ_1, λ_2, μ_1 and σ_2 as well as a protein not present in intact virions which was believed to be formed by cleavage of μ_2. (More recently Both, Lavi and Shatkin have reported the finding of additional reovirion structural proteins[10].) The ratio of RNA to protein increases as a consequence of the conversion to SVPs, consequently the buoyant density of SVPs is higher than that of reovirions (for a review on reovirus see ref. 11).

Reovirus cores which are produced by partial digestion of reovirions with chymotrypsin[12,13] and SVPs isolated from infected cells[7] contain an associated RNA dependent RNA polymerase (transcriptase) activity. When incubated in the presence of all four ribonucleoside triphosphates both types of particles catalyze the synthesis of reo mRNAs. Each of these mRNAs is transcribed from a different segment of the reovirus genome. The ten reo mRNAs fall into three size classes according to sedimentation velocity: large (mRNAs l_1, l_2, l_3), medium (mRNAs m_1, m_2, m_3) and small (mRNAs s_1, s_2, s_3, s_4). Furthermore, reovirus cores contain enzymes which attach to the 5' end of each of the reo mRNAs a guanylate residue (in the 3' to 5' direction), and other enzymes which catalyze the transfer of methyl residues from S-adenosyl-methionine (SAM) to the 7 position of the above noted 5' terminal guanylate residue and to the 2' position of the adjacent guanylate residue[14,15,16].

The data in this communication concern the formation and characteristics of SVPs in interferon-treated and control L cells. The data are, in general, in accord with those of Wiebe and Joklik[6] in not revealing a readily detectable effect of interferon on the early phase of reovirus replication. We find, however, that the accumulated products of RNA synthesis promoted by SVPs isolated from control cells (SVP_{CON}) and tested *in vitro* consist mainly of full size reo mRNA molecules whereas those whose synthesis was promoted by SVPs isolated from interferon-treated cells

(SVP_{INT}) consist mainly of shorter products. These and other results indicate that a nuclease may be associated with the SVP_{INT} preparation. However, premature termination of transcription could also account for these results and cannot be ruled out as a contributing factor at this time.

A nuclease activity associated with a membrane fraction from chick embryo cells which were treated with a partially purified interferon preparation has been reported by Marcus, Terry and Levin[17]. Furthermore Spandidos, Taylor-Papadimitriou and Georgatsos have reported an increase in an acid DNase activity in extracts of chick embryo fibroblasts which were treated with a partially purified preparation of interferon[18].

MATERIALS AND METHODS

Buffers and chemicals

PBS: 10 mM potassium phosphate (pH 7.4), 135 mM NaCl, 2.7 mM $MgCl_2$;
Buffer A: 10 mM Tris Cl (pH 7.6), 150 mM KCl, 1 mM $MgCl_2$, 0.5% Triton X-100 (Packard);
Buffer B: 10 mM Tris Cl (pH 7.5), 135 mM NaCl, 15 mM KCl, 2 mM $MgCl_2$;
Buffer C: 10 mM Tris Cl (pH 8.0), 10 mM NaCl, 0.5 mM $MgCl_2$;
Buffer D: 10 mM Tris Cl (pH 7.5), 10 mM NaCl, 1.5 mM $MgCl_2$;
Buffer E: 10 mM Tris Cl (pH 7.5), 10 mM NaCl, 1 mM EDTA.
All radioactive chemicals were obtained from New England Nuclear.

Interferon

A partially purified mouse interferon preparation was obtained by infecting mouse Ehrlich ascites tumor (EAT) cells with Newcastle disease virus and purifying the interferon produced according to the procedure of Weideli *et al.* (in preparation). The sp. act. of the interferon preparation was determined by a plaque reduction assay with vesicular stomatitis virus (VSV) according to Vassef *et al*[3]. The interferon units used throughout the paper are VSV plaque reduction units unless otherwise indicated. One such unit corresponds to about 10 NIH mouse reference standard units. The sp. act. of the two interferon preparations used was, as expressed in NIH mouse reference standard units/mg protein: I) 3.2×10^3 and II) 6.7×10^7.

Cell culture and virus preparations

The growth of L cells (mouse L 929 fibroblasts) and the growth and purification of reovirus (type 3, the Dearing strain) were as described by Graziadei and Lengyel[19]. Reovirus with [^{3}H] or [^{14}C]-labeled RNA was

obtained by infecting L cells in suspension culture supplemented with either 30 μCi/ml of [^{3}H] uridine (sp. act. 47.2 Ci/mmole) or 1 μCi/ml of [^{14}C] uridine (sp. act. 400 mCi/mmole). For this purpose L cells were cultured in suspension in growth medium (Eagle's MEM) supplemented with 5% fetal calf serum (previously dialyzed against PBS). Reovirus with [^{3}H] or [^{14}C]-labeled protein was prepared similarly except that the L cell cultures contained 1/4 of the usual concentration of amino acids and were supplemented with 20 μCi/ml of a [^{3}H]-labeled amino acid mixture or 10 μCi/ml of a [^{14}C]-labeled amino acid mixture. Reovirus with [^{32}P]-labeled-RNA was obtained by infecting L cells in monolayer cultures in phosphate-free growth medium supplemented with 7% fetal calf serum (previously dialyzed against phosphate-free Earle's balanced salt solution[20]) and 25 μCi of carrier-free [^{32}P] inorganic phosphate/ml.

Treatment of cells with interferon and infection with reovirus

Suspension cultures of L cells (5 to 6.5 x 10^{5} cells/ml of growth medium supplemented with 7% fetal calf serum) were exposed to interferon for 17 to 24 hours. In every experiment a portion of the culture was not exposed to interferon and served as a control. In both the interferon-treated and control cultures the number of cells doubled during this time period. Prior to infection the cell cultures were concentrated to 10^{7} cells/ml by centrifugation and resuspension in fresh medium of the same composition. Reovirus inoculum was added at the multiplicity of infection (moi: ratio of plaque forming units of virus to cells) indicated in each experiment and the cultures were incubated at 0-4°C for 1-3 hours to allow for the adsorption of virus to cells. The cells were sedimented by centrifugation, resuspended in fresh medium at a concentration of 10^{6} cells/ml and incubated at 37°C for the times indicated in each experiment. The yield of reovirus in a single growth cycle was determined in both the interferon-treated and control cells in each experiment by assaying the number of plaque forming units/ml in each culture after incubation at 37°C for 18 to 23 hours post adsorption. The single cycle yield reduction is defined as $\frac{V.Y._{CON} - V.Y._{INT}}{V.Y._{CON}} \times 100$ where $V.Y._{CON}$ is the virus yield in the control culture and $V.Y._{INT}$ is the virus yield in the interferon-treated culture.

Adsorption of reovirus to interferon-treated and control cells (to Table 1)

L cells were treated with 180 units of interferon I/ml of suspension culture for 21 hours. Control and interferon-treated cells were infected with [^{3}H] RNA-labeled reovirus (moi 9) at 0-4°. After 3 hours the cells were sedimented by centrifugation, washed twice with cold medium, resuspended at a concentration of 10^{6} cells/ml in the same medium, but at 37°C, and incubated at 37°C. The amount of virus was determined in aliquots taken from a) the viral inoculum added to the cultures, b) the supernatant fraction obtained by sedimenting the cells from the culture by centrifugation at the end of the period of adsorption, c) the first and d) second washing fluid and e) the supernatant fraction obtained as in (b) from the culture after 3.5 hours of incubation at 37°. The volume of the aliquot taken from (a) was the volume of viral inoculum added to 2 x 10^{5} cells. The size of all subsequent aliquots was the volume exposed to 2 x 10^{5} infected cells. Radioactivity in the aliquots was determined by counting in Bray's scintillation fluid and infectious virus by the plaque assay.

Time course of the conversion of parental reovirions into SVPs in interferon-treated and control L cells (to Fig. 1)

L cells were treated with 18 units of interferon I/ml of suspension culture. The interferon-treated cells were infected with [^{14}C] RNA-labeled reovirus (1.8 x 10^{7} pfu/10^{3} cpm) and the control cells with [^{3}H] RNA-labeled reovirus (3.45 x 10^{6} pfu/10^{3} cpm) both at an moi of 30. Aliquots were taken from both cultures after 30 minutes, 1 hour, 2 hours and 3.5 hours of incubation at 37°C. The cells in the aliquots were sedimented by centrifugation, resuspended in buffer A at 10^{8} cells/ml and incubated at 0-4°C for 15 minutes with occasional agitation on a Vortex mixer. Thereafter sodium deoxycholate was added from a 10% (w/v) solution, giving a final concentration of 0.5% (w/v) and the incubation continued in a similar manner for 10 minutes. The nuclei were removed from the resulting lysate by centrifugation at 725xg for 5 minutes. Aliquots of the resulting supernatant fractions containing equal amounts of radioactive material (in cpm) from the interferon-treated and the corresponding control preparations were combined. [^{32}P]-labeled reovirus was added as a density marker. The resulting mixtures were layered on 0.2 ml of a 20% sucrose solution in buffer A on top of a preformed CsCl gradient (1.32 to 1.41 gm/cm^{3}) in buffer A. The samples were centrifuged at 36,000 rpm in the SB283 rotor of the IEC B60 ultracentrifuge at 2° for 22 hours. The

gradients were fractionated and the buoyant density of several fractions was determined by refractometry. The radioactivity of each fraction was determined by counting in Bray's scintillation fluid. The buoyant densities of the labeled virus preparations which were used as inoculum were also determined.

Comparison of the double-stranded RNA and protein composition of reovirus SVPs from interferon-treated and control cells: analysis by electrophoresis in polyacrylamide gels (to Figs. 2 and 3)

1) Processing of viral samples: Viral precipitates were incubated in a solution of 6 M urea, 4% sodium dodecyl sulfate (SDS), 2% mercaptoethanol at room temperature for 1 hour. Thereafter the samples for double-stranded RNA analysis were further incubated at 45° for 5 minutes and those for protein analysis at 100° for 3 minutes. Aliquots containing equal amounts of radioactivity (in cpm) from interferon-treated cells (^{14}C-labeled material) and from control cells (^{3}H-labeled material) were combined and analyzed on the same gel. Aliquots containing equal amounts of radioactivity (in cpm) were also combined from the [^{3}H]-labeled and [^{14}C]-labeled reovirus preparations which were used as inoculum, and were similarly processed for gel electrophoresis.

2) Gel electrophoresis: 0.6 cm x 18 cm polyacrylamide gels were prepared essentially as described by Zweerink and Joklik[21]. 9% acrylamide gels were used for RNA analysis and 10% gels for protein analysis. Both types were prepared with 0.1% NNN'N' tetramethyleneethylenediamine, 0.08% ammonium persulfate, 0.3% NN' bis acrylamide, and 6 M urea in electrophoresis buffer (62 mM sodium phosphate pH 6.7, 20 mM EDTA, 0.1% SDS). Electrophoresis was performed at room temperature at 6 mA/gel for 78 hours in the case of double-stranded RNA analysis and 41 hours in the case of protein analysis. The gels for double-stranded RNA analysis were sliced into 0.6 mm thick slices, those for protein analysis into 0.8 mm thick slices. Each slice was solubilized in 0.5 ml of 90% NCS solubilizer (Amersham/Searle) and counted in 5 ml of a toluene-based scintillator.

Assay of the activities of the transcriptase and RNA methylase enzymes associated with reovirus SVPs isolated from interferon-treated and control cells (to Figs. 4 and 5)

1) Isolation of SVPs: Cells treated with 70 units of interferon II/ml of suspension culture and control cells were infected with reovirus at an moi of 60. The viral inoculum used had been supplemented with a trace amount (ca. 40,000 cpm/culture) of [^{14}C] RNA-labeled reovirus. Viral

adsorption was allowed to take place at 4° for 1 hour. After removal of the unadsorbed virus and incubation of the culture at 37° for 2.5 hours the cells were sedimented by centrifugation. All subsequent steps were carried out at 4° unless otherwise specified. The cell pellet was washed twice with buffer B, was resuspended in buffer C at a concentration of 4.7×10^7 cells/ml and was incubated for 15 minutes with agitation on a Vortex mixer. Subsequently the suspension was supplemented with Nonidet P40 (Shell International Chemical Co.) to a final concentration of 1% (v/v) and incubation was continued for 15 minutes. Thereafter sodium deoxycholate was added to a final concentration of 0.5% (w/v) and the incubation continued for an additional 15 minutes. The nuclei were sedimented from the resulting lysate by centrifugation at 7000xg for 10 minutes. The supernatant fractions were layered on discontinuous gradients consisting of 9 ml of CsCl solution (density 1.455 gm/cm^3)(lower layer) and 2 ml of a 20% sucrose solution (upper layer), both in buffer C. Upon centrifugation in the SW25 rotor (Spinco) at 22,000 rpm for 2 hours the SVPs were concentrated as a band on the CsCl layer. The SVPs were collected, diluted in a solution of CsCl in buffer C (density 1.35 gm/cm^3) and centrifuged to equilibrium in the SB283 rotor of the IEC B60 ultracentrifuge at 36,000 rpm at 2°C for 42 hours. The SVPs with buoyant densities between 1.385 and 1.405 gm/cm^3 were collected and dialyzed against buffer D. The sp. act. of the SVPs recovered from control cells (SVP_{CON}) was 3,750 cpm/A_{260} unit, those from interferon-treated cells (SVP_{INT}) 3,550 cpm/A_{260} unit.

2) Assay of the transcriptase activity of SVPs (to Fig. 4A): The composition of the reaction mixture was similar to that reported by Shatkin[14] and Faust and Millward[15]. 200 µl reaction mixtures contained 70 mM Tris Cl (pH 8.0), 150 mM KCl, 7.5 mM magnesium acetate, 2.5 mM phosphoenolpyruvate, 2 mM ATP, 2 mM CTP, 2 mM GTP, 0.4 mM [^{3}H] UTP (25 µCi), 3.9 international units of pyruvate kinase and 0.192 A_{260} unit of SVP_{CON} or SVP_{INT} or reovirions and if so indicated 14 µM SAM. The reaction mixtures were incubated at 44°C. 20 µl aliquots were taken at the times indicated in Fig. 4A. Cold trichloroacetic acid-insoluble radioactive material (i.e., RNA) was assayed according to the method of Bollum[22].

3) Assay of the RNA methylase activity of SVPs (to Fig. 4B): The composition of the reaction mixtures was the same as in the assay of transcriptase activity except that 0.086 A_{260} unit of SVP_{CON} or SVP_{INT} or reovirions were used in 100 µl reaction mixtures, the concentration of UTP (unlabeled) was 2 mM, the amount of pyruvate kinase was 1.85

international unit, and 0.36 μM [^{3}H] methyl-labeled SAM was added (4.2 μCi). 15 μl aliquots were taken at the times indicated in Fig. 4B. The radioactivity of the RNA was assayed according to published procedures[23].

4) Size distribution of the products synthesized in vitro by the transcriptase and methylase enzymes associated with SVPs isolated from interferon-treated and control cells (to Fig. 5): 40 μl aliquots were taken from the reaction mixtures designated as SVP_{CON} and SVP_{INT} in Fig. 4A after incubation at 44°C for 63 minutes. (For details see the section on "Assay of the transcriptase activity etc." and Fig. 4A.) Each aliquot was supplemented with 160 μl of buffer E at 4°. The resulting solution was extracted by shaking at 4° for 30 minutes with an equal volume of phenol saturated with buffer E containing 0.1% SDS. The resulting phenolic phase was extracted with an equal volume of buffer E and the pooled aqueous phases were reextracted with phenol saturated with buffer E. The aqueous phase was then extracted with ether and heated at 59° for 2 minutes. The resulting solutions, supplemented with [^{14}C]-labeled L cell rRNA and tRNA serving as sedimentation markers, were applied to the top of a linear 7 to 25% sucrose gradient in buffer E and centrifuged at 36,000 rpm in the SW40 rotor (Spinco) at 2°C for 17 hours. Fractions were collected and cold trichloroacetic acid-insoluble radioactive material was determined in each according to the filter paper disc method of Bollum[22] (to Fig. 5A).

The reactions described in Fig. 4B (incorporation of [^{3}H] methyl residues) were repeated with reaction mixtures identical in composition to those designated there as SVP_{CON} + SAM and SVP_{INT} + SAM. 90 μl aliquots were taken after 63 minutes of incubation and processed for sucrose gradient analysis as in Fig. 5A. Centrifugation was in the SB283 rotor of the IEC B60 ultracentrifuge at 40,000 rpm for 13 hours at 2°. The amount of radioactive material in the fractions of the gradients was determined as in Fig. 5A (to Fig. 5B).

RESULTS

Adsorption and conversion of parental reovirions to SVPs

The data in Table I reveal no difference in the extent of adsorption of reovirus to interferon-treated and control L cells. The extent of adsorption appeared greater when assayed by the number of plaque forming units than by the amount of labeled virus which adsorbed to cells. This apparent discrepancy in the extent of adsorption might indicate that not all virus particles were infectious.

The assay of the conversion of reovirions to SVPs, shown in Fig. 1,

Table 1

Adsorption of reovirus to interferon-treated and control cells

		Amount of reovirus			
		cpm/aliquot		pfu/aliquot	
		CON	INT	CON	INT
a.	Viral inoculum	1875	1875	1.8×10^6	1.8×10^6
b.	Supernatant fraction at the end of the adsorption period	491	528	3.4×10^5	3.7×10^5
c.	First washing fluid	105	147	3.4×10^3	5.4×10^3
d.	Second washing fluid	106	82	5.3×10^3	2.6×10^3
e.	Supernatant fraction after 3.5 hours of incubation	115	120	9.0×10^2	1.1×10^3
f.	b+c+d+e	817	877	3.5×10^5	3.8×10^5
g.	% of virus adsorbed f/a	56%	53%	81%	79%

The single cycle yield reduction in this experiment was 96%. CON refers to the control culture, INT to the interferon-treated culture. For details see the relevant section in MATERIALS AND METHODS.

was based on the difference in buoyant density between reovirions and SVPs. This change in density was followed by a) infecting interferon-treated cells and control cells with RNA-labeled reovirus, b) preparing cell extracts at different times after virus adsorption and c) centrifuging the cell extracts through preformed CsCl density gradients to determine the buoyant density of the labeled virus particles. [^{3}H] RNA-labeled reovirions were used for following the conversion in control cells and [^{14}C] RNA-labeled reovirions for following it in interferon-treated cells.

The gradient profiles in Fig. 1A indicate a slightly broader density distribution in the [^{14}C] RNA-labeled reovirus preparation than in the [^{3}H] RNA-labeled reovirus preparation. Although the cause of this difference is unclear, the difference did not affect the outcome of the experiments which when repeated with virus preparations of identical density distribution gave the same results (data not shown).

The curves in Fig. 1B to 1E indicate that there was no detectable difference in the rate or extent of conversion of parental reovirions to SVPs when comparing interferon-treated and control cells. The beginning

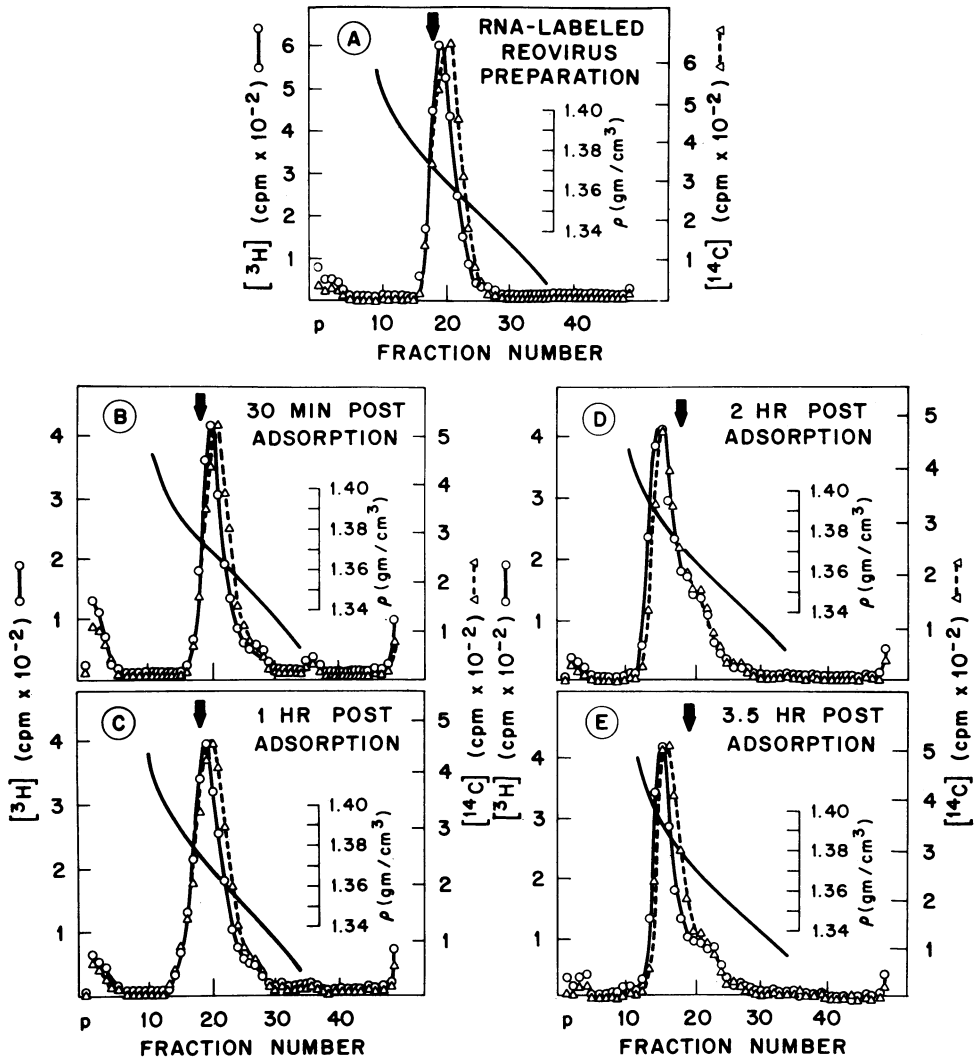

Fig. 1. Time course of the conversion of parental reovirions to SVPs in interferon-treated and control L cells

The buoyant densities of the labeled reovirus preparations used to infect cells were determined by equilibrium centrifugation in CsCl density gradients (A). The conversion of the RNA-labeled virions to SVPs in the infected cells was assayed by determining the buoyant density of radioactive material extracted from cells at various times after adsorption (B) to (E). The single cycle yield reduction in this experiment was 70%. Similar results were obtained in a second experiment in which the single cycle yield reduction was 96%. In this case the interferon-treated culture received 180 units of interferon I/ml of culture. (o——o), [^{3}H] RNA-labeled reovirus used as inoculum for control cells; (Δ---Δ), [^{14}C] RNA-labeled reovirus used as inoculum for interferon-treated cells. The arrows indicate the position in the gradients of [^{32}P]-labeled reovirions which served as a density marker (density 1.374 gm/cm^{3}). For details see the relevant section in MATERIALS AND METHODS.

of the conversion occurred between 1 and 2 hours after adsorption, and the process seemed to be complete by 3.5 hours after the adsorption period.

The distribution of radioactivity in polyacrylamide gels, shown in Fig. 2, reveals no significant difference in the double-stranded RNA composition between SVP_{CON} and SVP_{INT}. The distribution shown in Fig. 3A reveals that the protein composition of the [^{3}H] protein-labeled reovirus preparation used to infect untreated cells and that of the [^{14}C] protein-labeled reovirus used to infect interferon-treated cells were indistinguishable. The distribution in Fig. 3B indicates no significant difference in protein composition between SVP_{CON} and SVP_{INT}.

It is clear that the SVPs contain the major structural proteins λ_1, λ_2 and σ_2. The identification of the three medium sized proteins in the SVPs is less obvious. However, the gel electrophoretic analysis of the proteins of persistent reovirions (i.e. those which failed to convert to SVPs in infected cells and which had a buoyant density between 1.355 and 1.371 gm/cm^3) revealed four proteins with intermediate mobility (data not shown). Two of these predominated and corresponded in mobility to μ_1 and μ_2. The other two proteins, which are not present in the reovirus preparations shown in Fig. 3A, occurred in much smaller quantities and corresponded in mobility to the two protein peaks centered around slice number 76 or 85 in Fig. 1B. It is conceivable that these latter two proteins may be the products of cleavage of μ_1 and μ_2 and may appear in the persistent reovirion preparation either as a result of incomplete conversion of reovirions to SVPs or from contamination of the persistent reovirions with SVPs. If that were the case then the peaks of intermediate size viral protein in Fig. 3B may correspond, in order of increasing size, to the cleavage product of μ_2 (as proposed by Zweerink, McDowell and Joklik[24]) a cleavage product of μ_1, and a residual amount of μ_2 (and possibly μ_1). The presence of μ_2 (and μ_1) in the SVP preparations may result from a portion of these proteins which were not cleaved during the conversion to SVPs or from contamination by persistent reovirions. Estimates of molecular weights of these polypeptides are in accord with this possibility, that both μ_1 and μ_2 are cleaved during the conversion of reovirions to SVPs. Moreover, since μ_2 is derived from μ_1, a common mode of cleavage for the two proteins during the conversion to SVPs is imaginable.

In vitro synthesis and methylation of reo mRNAs by SVP_{CON} and SVP_{INT}

We also compared the enzymatic activities of SVP_{CON} and SVP_{INT} in

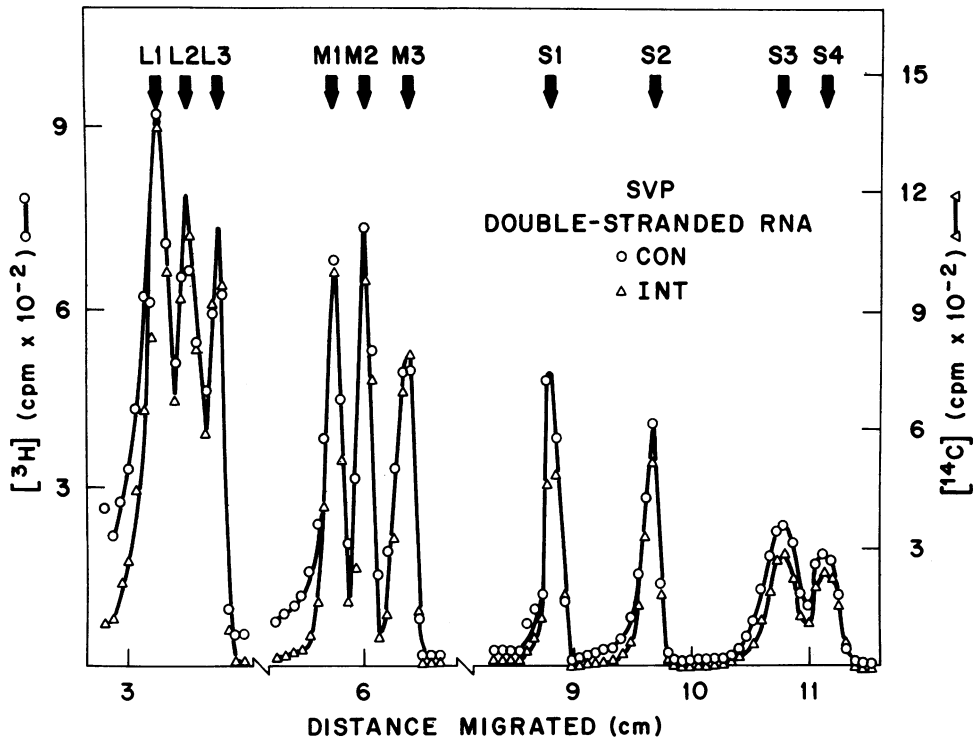

Fig. 2. Comparison of the double-stranded RNA composition of SVPs from interferon-treated and control cells: Analysis by electrophoresis in polyacrylamide gels

Interferon-treated cells which had been exposed to 180 units of interferon I/ml of suspension culture were infected with [^{14}C] RNA-labeled reovirus (5 x 10^7 pfu/10^3 cpm), and control cells with [^{3}H] RNA-labeled reovirus (7.5 x 10^6 pfu/10^3 cpm), both at an moi of 30. The infected cells were harvested 5.5 hours after the end of the adsorption period. Cell extracts were prepared and then fractionated (without combining the extracts from interferon-treated and control cells) as described in the section on the "Time course of the conversion etc." in MATERIALS AND METHODS, except that no ^{32}P-labeled reovirus was added as density marker. The fractions containing the SVPs (buoyant density between 1.384 and 1.402 gm/cm^3) were pooled separately from each gradient, purified reovirus (unlabeled) was added as carrier, and the SVPs and the virus were precipitated with 10 volumes of 80% acetone at -20°C. The processing of the viral samples and the analysis of the double-stranded RNA by gel electrophoresis were as described in the relevant sections in MATERIALS AND METHODS. The arrows indicate the positions to which the double-stranded RNA segments migrated (left to right). (o——o), SVP double-stranded RNA from control cells; (Δ——Δ), SVP double-stranded RNA from interferon-treated cells. The single cycle yield reduction in this experiment was 96%.

vitro. For this purpose SVPs were isolated from extracts of infected cells by equilibrium density gradient centrifugation in CsCl gradients.

No difference was found in the rate of viral RNA synthesis promoted by SVP_{CON} and SVP_{INT} (Fig. 4A). The addition of the methyl donor SAM to

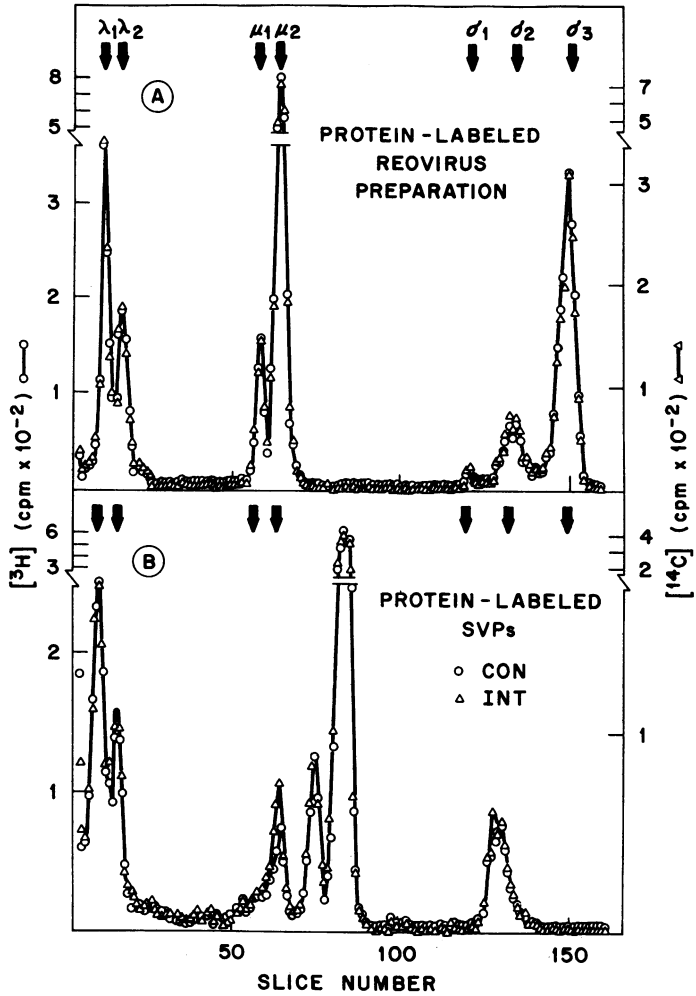

Fig. 3. Comparison of the protein composition of SVPs from interferon-treated and control cells: Analysis by electrophoresis in polyacrylamide gels

Interferon-treated cells which had been exposed to 180 units of interferon I/ml of suspension culture were infected with [^{14}C] protein-labeled reovirus (1.1 x 10^7 pfu/10^3 cpm), and control cells with [^{3}H] protein-labeled virus (2.5 x 10^7 pfu/10^3 cpm) both at an moi of 30. The infected cells were harvested 3.5 hours after the end of the adsorption period. Cell extracts were prepared and then fractionated, (without combining the extracts from interferon-treated and control cells) as described in the section on the "Time course of the conversion etc." in MATERIALS AND METHODS. The fractions containing the SVPs (buoyant density between 1.385 and 1.405 gm/cm^3) were pooled separately from each gradient and precipitated with 10 volumes of 80% acetone at -20°C. The processing of the viral precipitates and the analysis by gel electrophoresis of the protein composition of these as well as those of a mixture of [^{3}H] and [^{14}C] protein-labeled reovirions which had been used as inoculum was as described in the sections on "Processing of viral samples" and "Gel electrophoresis" in MATERIALS AND METHODS. Polyacrylamide gel electrophoresis of (A) proteins from a mixture of [^{3}H] and [^{14}C]-labeled reovirions used as inoculum and (B) the proteins from the mixed pooled fractions of SVPs from infected control (o——o) and interferon-treated (Δ——Δ) cells. The arrows in (A) and (B) indicate the positions to which the major reovirion structural proteins migrated (left to right). The single cycle yield reduction in this experiment was 96%.

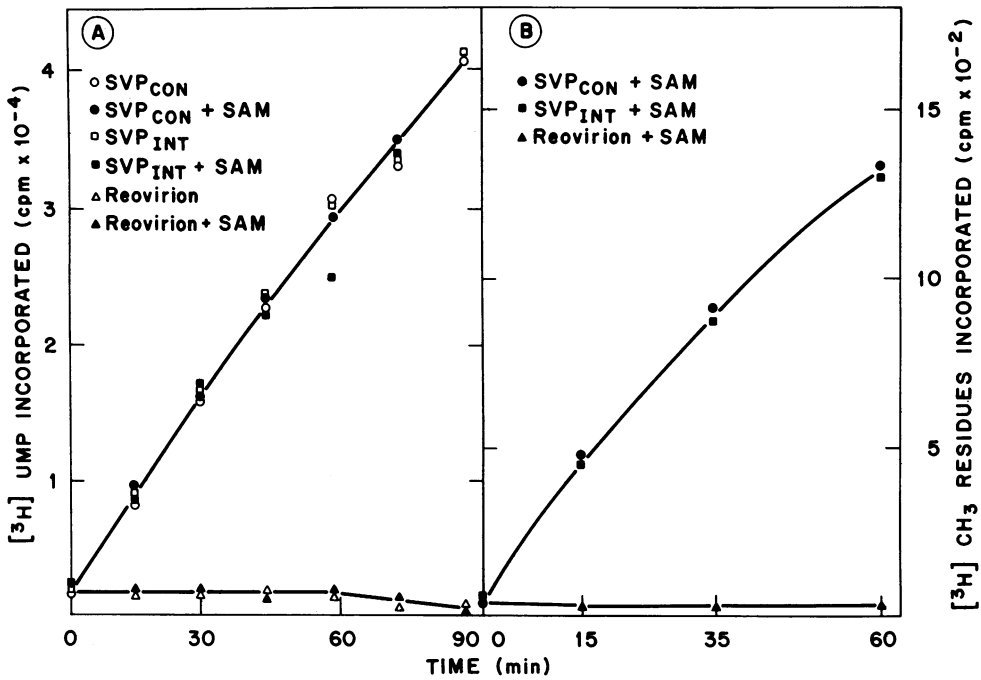

Fig. 4. Assay of the activities of (A) the transcriptase and (B) the methylase enzymes associated with SVPs isolated from interferon-treated and control cells

The single cycle yield reduction in this experiment was 92%. For details see the relevant section in MATERIALS AND METHODS.

the reaction mixtures did not affect the rate of RNA synthesis. Furthermore, no difference was detected in the rate of incorporation of [^{3}H] methyl residues, presumably into RNA, by SVP$_{CON}$ and SVP$_{INT}$ (Fig. 4B). As expected, no incorporation of substrate was detected in reaction mixtures in which intact reovirions were substituted for SVPs (Fig. 4A and B). These data seem to indicate that the conversion of parental virions to SVPs in interferon-treated cells results in particles as active in synthesizing viral RNA as those found in control cells.

The size distributions of the [^{3}H] uridylate residue-labeled RNAs synthesized by SVP$_{CON}$ and SVP$_{INT}$ were compared by centrifugation through sucrose gradients (Fig. 5A). The sedimentation pattern of mRNA synthesized by SVP$_{CON}$ *in vitro* (continuous line in the figure) reveals that, as expected, the RNAs fell into three size classes. The amount of RNA in each size class was similar. The ratio of sizes of the three reo mRNA size

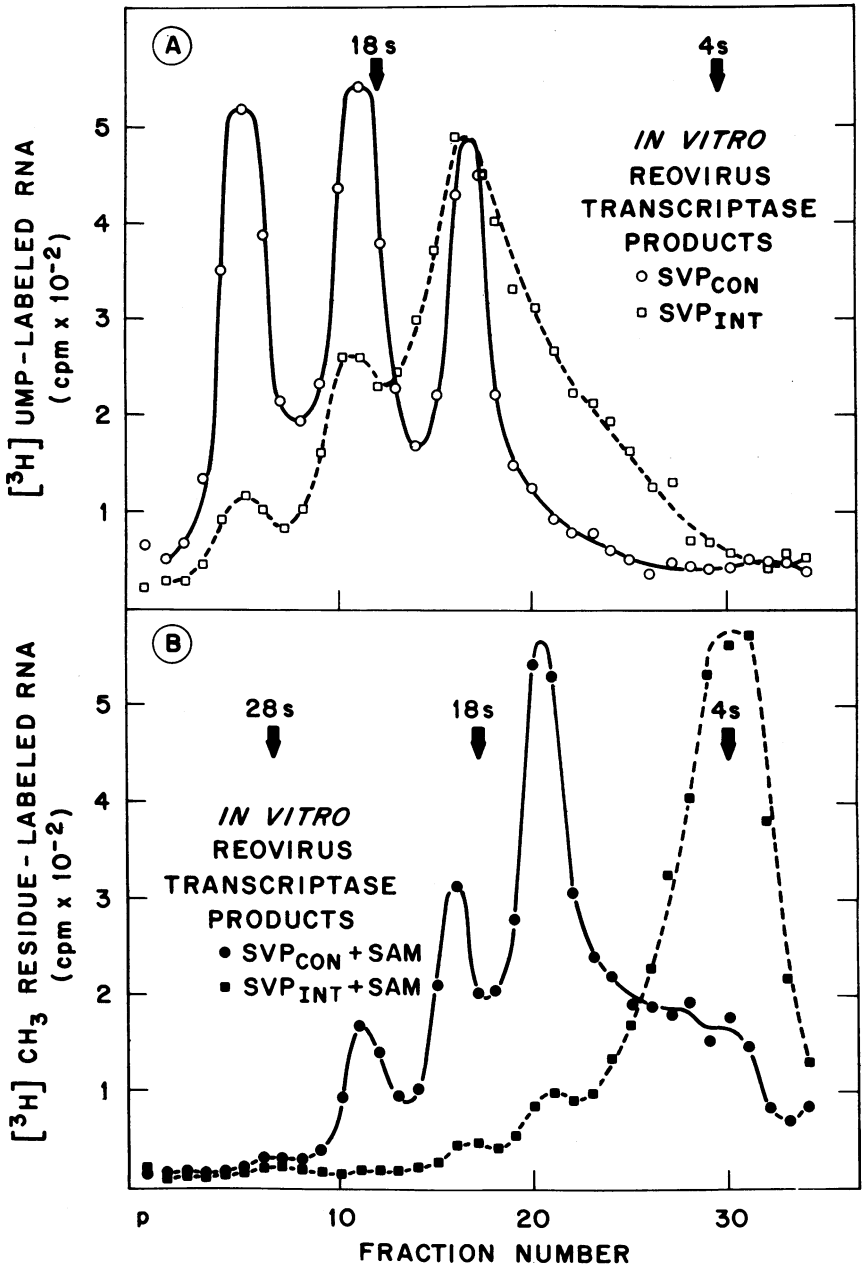

Fig. 5. Size distribution of the products synthesized in vitro by the transcriptase and methylase enzymes associated with SVPs isolated from interferon-treated and control cells: Analysis by sucrose gradient centrifugation of: (A) reoviral RNA labeled with [^{3}H]-uridylate residues; (B) reoviral RNA labeled with [^{3}H] methyl residues

The single cycle yield reduction in this experiment was 92%. For details see the section on the "Size distribution etc." in MATERIALS AND METHODS.

classes is close to 4:2:1. Thus, the fact that they are produced by SVP_{CON} in close to equal amounts in terms of mononucleotides incorporated into RNA is an indication that the molar ratio of the products is about 1:2:4.

The sedimentation pattern of mRNAs synthesized by SVP_{INT} (broken line in the figure) indicates that the products also fell into three size classes. However, there was much less RNA in the two larger size classes of reo mRNAs and a significant amount of RNA products sedimented slower than the smallest size class indicating the accumulation of products smaller than full size reo mRNA molecules when the synthesis was promoted by SVP_{INT}.

The sedimentation pattern of RNAs synthesized by SVP_{INT} and SVP_{CON} *in vitro* is also shown in Fig. 5B. The label in this case, however, was in [^{3}H] methyl residues transferred from SAM into *de novo* synthesized reo mRNA. The products of SVP_{CON} (continuous line in the figure) again fell into three size classes. The relative amounts of labeling in these were (in order of decreasing sedimentation velocity) about 1:2:4. This is the expected ratio since the molar ratio of products in the three size classes is 1:2:4 and two methyl residues are expected to be incorporated into each reo mRNA[13]. The sedimentation pattern of the products whose synthesis was promoted by SVP_{INT} (dashed line in the figure) reveals that the majority of these were substantially shorter than the smallest class of reo mRNAs and that only trace amounts corresponded in size to full size reo mRNAs.

DISCUSSION

The results presented reveal that the adsorption of reovirus and the conversion of parental reovirions to SVPs are not impaired in interferon-treated L cells. Furthermore, the double-stranded RNA and protein composition of the SVPs formed in interferon-treated and control cells are indistinguishable. These results make it probable that the block in reovirus reproduction in interferon-treated cells may occur in a later phase of the replicative cycle. The effect of interferon on a late phase of virus replication is also supported in studies with other viruses in which the infectivity of naked viral RNA was found to be impaired in interferon-treated cells (see e.g. refs. 25,26).

In agreement with Wiebe and Joklik[6] we found little if any difference between the capacities of isolated SVP_{CON} and SVP_{INT} to incorporate uridylate residues and methyl residues into *de novo* synthesized RNA.

A remarkable difference was found, however, in the size distribution between reo mRNAs made *in vitro* by SVP_{CON} and those by SVP_{INT}. SVP_{CON}

promoted the accumulation of full size reo mRNAs distributed about equally between the three mRNA size classes whereas SVP_{INT} promoted the accumulation mainly of smaller RNAs with a major portion of these being shorter than the smallest reo mRNAs. Similar results have been observed by W. Graziadei in experiments with SVPs obtained from cell lysates by centrifugation through 1 M sucrose (personal communication).

Preferential synthesis of the shorter classes of reo mRNAs by SVP_{INT}, although not ruled out as a contributing factor, could not account for the occurrence of RNA sedimenting slower than the smallest reo mRNAs. Premature termination of transcription and degradation of full size reo mRNA molecules could each account for the data. Our preliminary experiments revealed that, when tested *in vitro*, SVP_{INT} degraded added ribosomal RNA into trichloroacetic acid-insoluble products, whereas SVP_{CON} did not (data not shown). This finding indicates that an endonuclease is associated with our SVP_{INT} preparation. This endonuclease may be partially or entirely responsible for the results obtained. Possibly in line with the above observations is the finding that added reo mRNA is degraded faster in S30 extracts of interferon-treated Ehrlich ascites tumor cells than in comparable extracts of control cells (G. Brown, B. Lebleu, *et al.*, unpublished data).

The interferon preparation used in our experiments was only partially purified and was shown earlier to exhibit an endonuclease activity[27]. The relationship, if any, between interferon, the endonuclease activity in the interferon preparation, and the endonuclease activity in our SVP_{INT} preparation is under investigation.

Several investigators have reported an impairment of viral RNA accumulation as a possible site of interferon action[2,28-33]. Results from other laboratories however, are not in line with this conclusion (see e.g. refs. 34,35).

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