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Transcription of the Genomes of Type 1 and Type 3 Reoviruses

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The double-stranded ribonucleic acid (RNA) genome of type 1 reovirus was fragmented into three size classes on extraction, as has already been shown for reovirus type 3. The relative amounts and molecular weights of the three classes were the same for the two viruses. Cells infected with type 1 virus synthesized three classes of messenger RNA. Each class of messenger RNA hybridized exclusively with a denatured double-stranded RNA fragment of equivalent length, as had also been found for type 3 reovirus. The double-stranded RNA segments thus act as specific units for transcription of messenger RNA in the infected cells. In cells infected with type 3 reovirus, the three classes of messenger RNA are made in equal amounts throughout the course of multiplication. In contrast, cells infected with type 1 virus produced only half as much of the largest messenger RNA as they did of the other two classes at all times during the replicative cycle. This finding suggests that transcription of the largest segments of type 1 viral genome is restricted.

The genome of type 3 reovirus is a double-stranded ribonucleic acid (dsRNA) considered to have a minimal molecular weight of approximately 10^7 daltons (6, 10). Apparently, there are weak spots in the molecule since, on extraction from virions or infected cells by a variety of methods, the dsRNA is obtained in lengths which fall into three main size classes (2, 3, 7, 9, 17). These classes have molecular weights of approximately 0.8×10^6, 1.4×10^6, and 2.4×10^6 daltons (17). Hybridization tests on denatured RNA from the three classes, taken in all possible pairs, showed no homology to exist between them; the fragments of dsRNA therefore arise through disruption of the viral genome at specific points rather than by random fragmentation (17). For each class of dsRNA that can be isolated, a messenger RNA (mRNA) of similar length is produced in infected cells (1, 6, 17), and each of the three classes of mRNA hybridizes exclusively with its corresponding length of viral RNA (1, 17, 19). Thus, the various segments of double-stranded viral genome appear to act as individual templates for transcription of mRNA during the cycle of infection. In the present paper, it is shown that type 1 reovirus, although serologically distinct from type 3, is similar to type 3 virus in the above respects.

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A major problem arising from these observations is associating functions with each class of mRNA. One approach, which has been used here, is to examine the kinetics of synthesis of the three classes of mRNA in an effort to determine whether they may be synthesized at different times in the infected cell and could, therefore, be associated with early or late functions in the replicative process. It will be shown that, insofar as type 3 reovirus is concerned, all three classes of mRNA are synthesized in approximately equal amounts at early as well as late times during infection. With type 1 reovirus, the relative amounts of the three classes of mRNA synthesized also remained constant, although much less of the largest mRNA was made in the type 1 than in the type 3 system. Apparently, transcription of the largest double-stranded segments is markedly restricted in the type 1 system.

MATERIALS AND METHODS

Cells and virus. Suspension cultures of L cells were used for all experiments except one in which BHK21 cells were employed. The latter cells were obtained from T. Wiktor of The Wistar Institute. The medium was as previously described (10, 18). Cultures were infected by allowing virus, at a multiplicity of 20 to 30 plaque-forming units per cell, to adsorb for 2 hr at room temperature to cells in suspension at a concentration of 10^6 cells/ml. The cells were then centrifuged and resuspended to a concentration of 5×10^5/ml in medium; they were placed at 37 C and

actinomycin D was added to a concentration of 0.5 μg/ml. This time is regarded as time-zero.

Reovirus, type 3, was a large-plaque variant isolated for the present work from the Dearing strain originally obtained from P. Gomatos. Reovirus, type 1 (Lang strain), was obtained from J. Levy of the University of Pennsylvania.

Virus assay. Infectious virus was assayed by a plaque technique. Monolayer cultures of L cells in 60-mm diameter plastic petri dishes were drained of medium, and 0.2 ml of virus was applied. After 2 hr at 35 C to permit adsorption of virus, each monolayer was overlaid with 5 ml of medium containing 1% agar (Difco), 1% fetal calf serum, 30 units of nystatin per ml, and 100 μg of diethylaminoethyl dextran per ml. Diethylaminoethyl dextran had the effect of markedly increasing the final size of the plaques. After incubating the plates for 7 days at 35 C in a 5% CO_2-air atmosphere, the plaques were enumerated.

Quantitative hemagglutination tests with the use of bovine erythrocytes for type 3 virus and human type 0 erythrocytes for type 1 virus (4) were used routinely to assay virus yields from infected cultures and to detect cross-contamination of the two viruses in lysates and stocks. In addition, cross-contamination by type 3 virus was checked with antiserum prepared in rabbits against type 3 virus. At an appropriate dilution, this antiserum neutralized type 3 virus to 10^{-3} survival, whereas the titer of type 1 virus was reduced by only a few per cent. According to these tests, performed routinely throughout the present work, no cross-contamination of the two viruses occurred.

Preparation of virus-specific RNA. The extraction of dsRNA from purified virus or infected cells, and its separation into three fractions by chromatography on columns of methylated bovine albumin-kieselguhr (MAK), has been described (17). These fractions have been designated dsRNA-1, dsRNA-2, and dsRNA-3 in order of elution from the column, and they have molecular weights of 0.8×10^6, 1.4×10^6, and 2.4×10^6 daltons, respectively (17). Isolation of virus-specific single-stranded RNA (ssRNA), that is, virus-specific mRNA, from infected cells and its separation into three fractions by sucrose gradient sedimentation were done as described previously (17) except that the Macaloid treatment was omitted. Molecular weights of the three classes of ssRNA are 0.4×10^6, 0.7×10^6, and 1.2×10^6 daltons, and they have been designated ssRNA-1, ssRNA-2, and ssRNA-3 (17).

To obtain polyribosomes containing virus-specific mRNA (14), cells were infected with reovirus and 0.5 μg of actinomycin D was added per ml of culture at time-zero. Ten hours after infection, ^{3}H-uridine (1 μc/ml) was added. Cycloheximide, to a concentration of 10 μg/ml was added at 11.5 hr postinfection, and the cells were harvested 0.5 hr later. Addition of cycloheximide 0.5 hr prior to polyribosome extraction resulted in a large increase in polyribosomal material, as measured by optical density, and up to a twofold increase in the amount of virus-specific mRNA associated with polyribosomes. Accumulation of polyribosomes in mouse embryo cells by much lower concentrations of cycloheximide has been reported by Stanners (16). It has been shown that cycloheximide added at this point in the growth cycle of reovirus has no effect on the kinetics of virus-specific mRNA synthesis, although the formation of viral RNA itself is rapidly blocked (15, 18). After the above treatment, polyribosomes were extracted from the cells and analyzed by sedimentation through sucrose gradients as already described (14). Fractions representing the polyribosome region of the gradients were pooled, and RNA was released by the addition of sodium dodecyl sulfate (SDS) to a final concentration of 0.5%. After standing at room temperature for several minutes, the RNA was precipitated by addition of 2 volumes of ethyl alcohol and was stored at 4 C for 18 hr. The RNA was then centrifuged, dissolved in LTM buffer [0.14 M LiCl, 0.01 M tris(hydroxymethyl)aminomethane, *p*H 7.6, and 0.001 M $MgCl_2$] containing 0.5% SDS, heated to 70 C for 5 min to dissociate aggregates of RNA, and analyzed by sucrose gradient sedimentation.

Sucrose gradient sedimentation of RNA. A sample (0.05 to 0.2 ml) was layered over 5 ml of a 5 to 20% linear sucrose gradient, and the whole was centrifuged at 48,000 rev/min for 150 min. The tube was punctured at the bottom, and fractions were assayed for labeled RNA insoluble in 5% trichloroacetic acid and for RNA insensitive to the action of ribonuclease, by methods already described (17, 18). On some occasions, to be specified, fractions were collected from the sucrose gradient directly onto 2.4-cm glass-fiber filters placed in the bottom, of scintillation vials. Radioactivity determinations by liquid scintillation counting were done as described previously (17, 18).

Hybridization procedure and electrophoresis on acrylamide gels. The details of denaturation of double-stranded RNA with dimethyl-sulfoxide, hybridization of the denatured RNA with ssRNA, and analysis of the products by electrophoresis on acrylamide gel columns have been described (17, 19).

Kinetics of synthesis of ssRNA and dsRNA in infected cultures. The method for determining the overall course of synthesis of the two types of RNA was similar to that previously described (18). Cells were infected with reovirus type 1 or type 3 in the standard way. At intervals, a sample was withdrawn from each culture and an additional 2 μg of actinomycin D per ml was added. After 30 min, 1 μc of ^{3}H-uridine per ml was added. After 1 hr more, a known number of ^{14}C-uridine-labeled L cells was added to each sample, the whole was centrifuged, and the total RNA was extracted with phenol. The amount of trichloroacetic acid-insoluble and ribonuclease-sensitive RNA in each sample was determined, and the results were normalized to each other through the content of ^{14}C-labeled RNA. In parallel experiments, the amounts of ^{3}H-uridine incorporated into uninfected cells, under otherwise identical conditions, were measured and subtracted from the values obtained for the infected cultures.

RESULTS

The present study was primarily concerned with the time course of transcription of the viral genome in cells infected with either type 1 or type 3 reovirus. Before presenting the results bearing specifically on this question, however, we describe some comparative experiments to show that the two virus systems have a number of salient features in common.

Analysis of dsRNA from cells infected with reovirus type 1 and type 3. Double-stranded RNA extracted from cells infected with type 1 reovirus fragmented in the same way as type 3 viral RNA, as the following experiment showed. One culture of L cells was infected with type 1 virus and labeled with ^{3}H-uridine. A second culture was infected with type 3 virus and labeled with ^{14}C-uridine. At 20 hr after infection, the cells from the two cultures were centrifuged and combined, and the viral dsRNA extracted from them. This dsRNA was purified by adsorption to a MAK column and elution in a single narrow band with a steep sodium chloride gradient, as previously described (10). The RNA was then analyzed by electrophoresis on an acrylamide gel column with the results shown in Fig. 1. Classes dsRNA-1a, dsRNA-1b, dsRNA-2, and dsRNA-3 from type 3 reovirus separated from each other as described in a previous paper (17). ^{3}H-labeled dsRNA from type 1 virus migrated in an almost identical fashion to ^{14}C-labeled dsRNA from type 3 virus. In other experiments, cultures were infected either with type 1 or type 3 virus and labeled between 10 and 12 hr after infection. Double-stranded RNA extracted at 12 hr from those cultures and analyzed by electrophoresis gave patterns similar to those shown in Fig. 1. Evidently, the genomes of type 1 and type 3 vriuses are comprised of similar segments of dsRNA in the same relative amounts.

Single-stranded RNA from virus-specific polyribosomes. L cells infected with type 3 reovirus synthesize as much virus-specific single-stranded RNA as they do double-stranded progeny RNA (10, 18). Part, if not all, of this single-stranded RNA is attached to polyribosomes and has been characterized as virus-specific mRNA (14, 15, 18). The following experiment was done to determine the sedimentation properties of the mRNA associated with polyribosomes from cells infected with type 1 virus. Two cultures of L cells were infected, one with type 1 and the other with type 3 virus, and were labeled with ^{3}H-uridine between 10 and 12 hr postinfection. At the end of the period of labeling, polyribosomes were obtained from both cultures, and their RNA was extracted and analyzed by sucrose gradient sedimentation (Fig. 2).

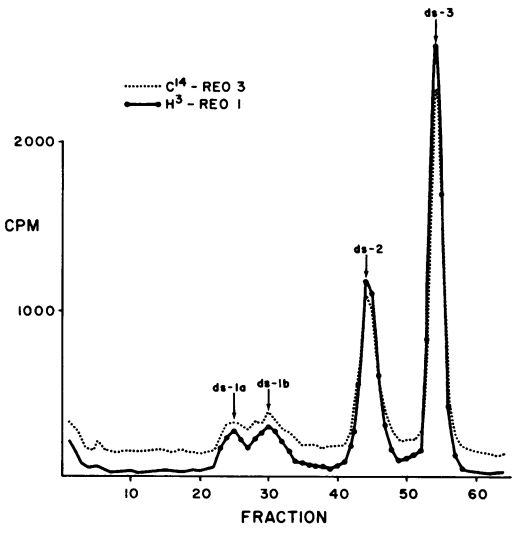

FIG. 1. *Electrophoresis on acrylamide gel of dsRNA isolated from cells infected with reovirus type 1 and reovirus type 3. Two 200-ml suspension cultures of L cells were separately infected with either type 1 or type 3 reovirus. At 6 hr postinfection, ^{3}H-uridine (0.5 μc/ml) was added to the type 1-infected culture and ^{14}C-uridine (0.05 μc/ml) was added to the type 3-infected culture. The cultures were centrifuged at 20 hr after infection, the cells were pooled, and dsRNA was extracted. After purification on a MAK column, the dsRNA was analyzed by electrophoresis on acrylamide gel. The gel was sliced, and the slices were assayed for radioactivity. The anode is to the left of the diagram.*

Messenger RNA from type 3 virus-induced polyribosomes was separated into three distinct fractions. Sedimentation coefficients of the three classes of single-stranded RNA are approximately 24.5*S*, 18.5*S*, and 14.0*S*; in accordance with our previous nomenclature, these fractions will be referred to as ssRNA-3, ssRNA-2, and ssRNA-1, respectively (17). Polyribosomes from type 1 virus-infected cells also yielded three classes of ssRNA with sedimentation rates similar to those found for type 3 mRNA. Although the three fractions of ssRNA from type 3 virus-infected cells were present in approximately equal amounts, the quantity of ssRNA-3 from type 1 virus-infected cells was strikingly less than that of ssRNA-2 or ssRNA-1.

ssRNA extracted directly from infected cells. The reduced yield of ssRNA-3 from polyribosomes of type 1 virus-infected cells may have resulted from selection against a part of this ssRNA which either attached poorly to polyribosomes or was not used as mRNA. As one means

of determining the cause, mRNA was extracted directly from infected cells and analyzed. One culture of L cells was infected with reovirus type 3 and a second with reovirus type 1. The first culture was labeled with ^{3}H-uridine and the second with ^{14}C-uridine between 10 and 12 hr postinfection. Both cultures were then centrifuged, the two pellets of cells were mixed, and the ssRNA was extracted with phenol (17) and analyzed by sedimentation through a sucrose gradient (Fig. 3).

Three classes of type 3 mRNA were present in approximately equal amounts, as was shown in Fig. 2 and previously (17). Three classes of type 1 mRNA were also found with sedimentation rates almost identical to those of the type 3 fractions. The large peak of labeled material at the top of the tube was comprised mainly of acid-soluble components. Moreover, all the material in fractions ssRNA-1, ssRNA-2, and ssRNA-3 was sensitive to the action of 2 μg of ribonuclease per ml for 30 min at 37 C (17), suggesting that it was single-stranded. It is again apparent, as it was in Fig. 2, that a relatively small amount of ssRNA-3 was induced by infection with type 1 reovirus.

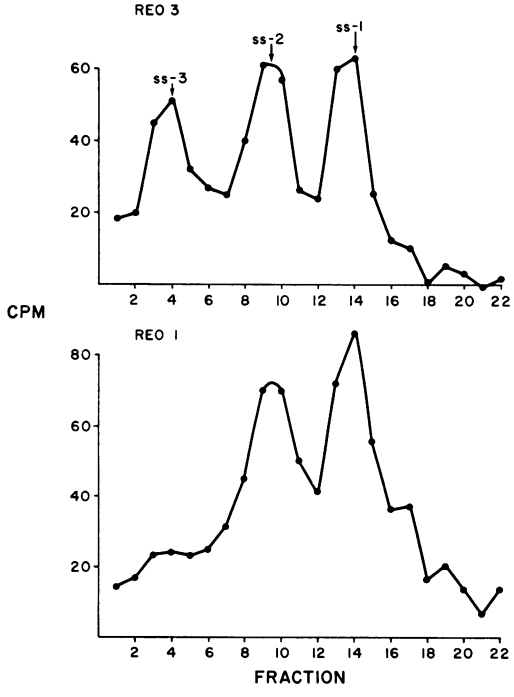

FIG. 2. *Sucrose gradient sedimentation analysis of ssRNA from polyribosomes of type 1 and type 3 virus-infected cells.*

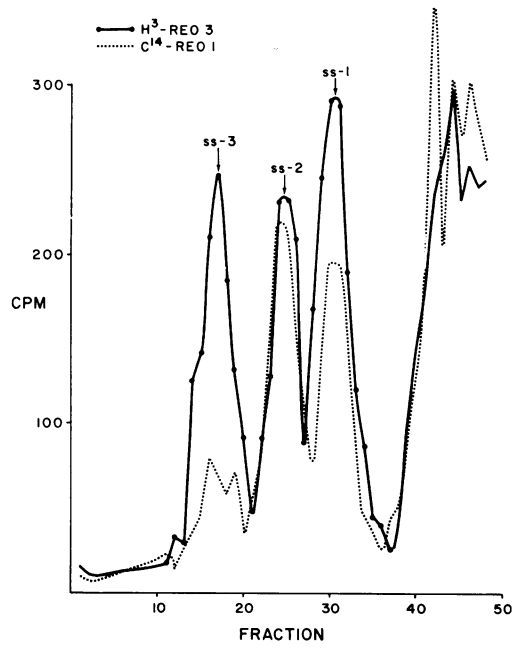

FIG. 3. *Sucrose gradient sedimentation analysis of ssRNA extracted directly from type 1- and type 3-infected cells. Two L-cell cultures were separately infected with type 1 or type 3 virus. At 9.5 hr postinfection, additional actinomycin D and cycloheximide (20 μg/ml) were added to each culture. After an additional 0.5 hr, ^{3}H-uridine (1.0 μc/ml) was added to the type 3-infected culture and ^{14}C-uridine (0.2 μc/ml) was added to the type 1-infected culture. At 12 hr postinfection, the cultures were centrifuged, the cells were pooled, and the ssRNA was extracted. The extracted RNA was sedimented through a sucrose gradient, and fractions were collected directly onto glass-fiber filters in scintillation vials for assay of radioactivity.*

Selectivity in transcription of mRNA from the the type 1 reovirus genome. The three classes of mRNA induced by type 3 virus infection have been found to be uniquely transcribed from segments of viral genome of corresponding length (1, 19). In this section, evidence is presented for a similar situation in the transcription of the type 1 reovirus genome.

The methods used have been described in detail (17, 19). Essentially, ssRNA labeled with ^{3}H-uridine was extracted from cells infected with type 1 reovirus and was separated by sucrose gradient sedimentation into ssRNA-1, ssRNA-2, and ssRNA-3. ^{14}C-labeled dsRNA was obtained from type 1 infected cells, adsorbed to a MAK column, and eluted as a mixture of the three dsRNA classes with a steep NaCl gradient (10). A sample of each ssRNA was added to a large

excess of dsRNA preparation; the mixture was denatured with dimethylsulfoxide (DMSO), and then reannealed (17, 19). The three reannealed samples were then analyzed by electrophoresis on columns of acrylamide gels (19); the results are shown in Fig. 4. Clearly, ssRNA-1 hybridized almost exclusively with denatured dsRNA-1, ssRNA-2 with dsRNA-2, and ssRNA-3 with dsRNA-3; they were, therefore, uniquely transcribed from the corresponding double-stranded segments during infection, as previously shown for reovirus type 3 (19). The similarity between the two types of virus holds even to the reduced homology of ssRNA-1 with the slower-moving fraction of dsRNA-1 (Fig. 4a). During each of these analyses, a small amount of label was left at the top of the gel column; the amount of this material varied from one analysis to another and probably resulted from the formation of imperfectly hybridized structures that do not migrate through the gel (Watanabe, *unpublished data*). In addition to showing the uniqueness of homology between a given class of ssRNA and a corresponding segment of viral double-stranded RNA, this result indicated that the relatively small quantity of type 1 ssRNA-3 could not be accounted for by some breakdown and appearance of the fragments in the ssRNA-1 or ssRNA-2 classes.

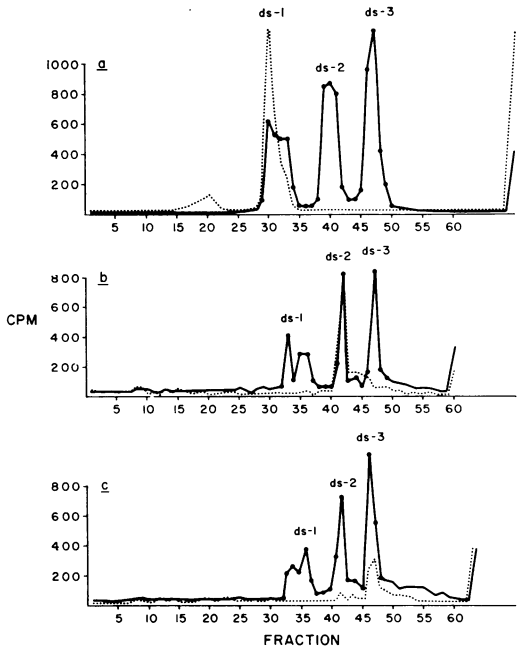

FIG. 4. *Electrophoresis on acrylamide gel of each class of type 1 ssRNA after hybridization with type 1 dsRNA. Single-stranded RNA was extracted from type 1-infected cells which had been labeled with ^{3}H-uridine from 10 to 12 hr postinfection, and the three fractions, ssRNA-1, ssRNA-2, and ssRNA-3, were separated by sucrose gradient centrifugation. Double-stranded RNA was obtained from type 1-infected cultures that had been labeled with ^{14}C-uridine from 6 to 20 hr postinfection, and was purified by chromatography on a MAK column. Each ssRNA fraction was separately annealed with the dsRNA, and the resulting product analyzed by electrophoresis on polyacrylamide gels. (a) ssRNA-1 hybridized with denatured dsRNA; (b) ssRNA-2 hybridized with denatured dsRNA; (c) ssRNA-3 hybridized with dsRNA.*

Kinetics of RNA synthesis in cells infected with reovirus types 1 and 3. The only difference so far discernible between type 1 and type 3 virus infection was the small amount of ssRNA-3 made in cells infected with type 1 virus. This observation could be explained if ssRNA-3 were a mRNA produced in larger amounts at another time in the infectious cycle, or if the overall course of RNA synthesis was much different in type 1 infected cells than in cells infected with type 3 virus. To settle this latter question, the kinetics of dsRNA and ssRNA synthesis were determined in cultures infected with one or other type of virus as described in Materials and Methods (Fig. 5).

Synthesis of ssRNA and dsRNA commenced in both cultures at approximately 5 hr after infection and pursued the same general course until 15 hr. Gross differences in the overall rates of synthesis of the two types of virus-specific RNA, therefore, did not account for the reduced amount of ssRNA-3 found in type 1-infected cells.

Rates of formation of the three classes of ssRNA in cells infected with type 1 or type 3 reovirus. So far in this paper, we have considered the analysis of ssRNA formed during one interval, between 10 and 12 hr after infection, at which time its synthesis is proceeding at close to the maximal rate (Fig. 5). Since the mRNA induced by infection with either type of virus can be readily separated into three size classes, however, there was an opportunity to determine the relative rates of synthesis of these classes at different times during infection.

To this purpose, one culture was infected with type 3 virus and another with type 1 virus. Samples were taken at intervals from each culture and labeled with ^{3}H-uridine for 2 hr, as described in the legend to Fig. 6. Immediately after each labeling period, the ssRNA was extracted and analyzed by centrifugation through sucrose gradients. The results are shown in Fig. 6 for the two cultures. The total area under each sedimentation profile was determined and normalized

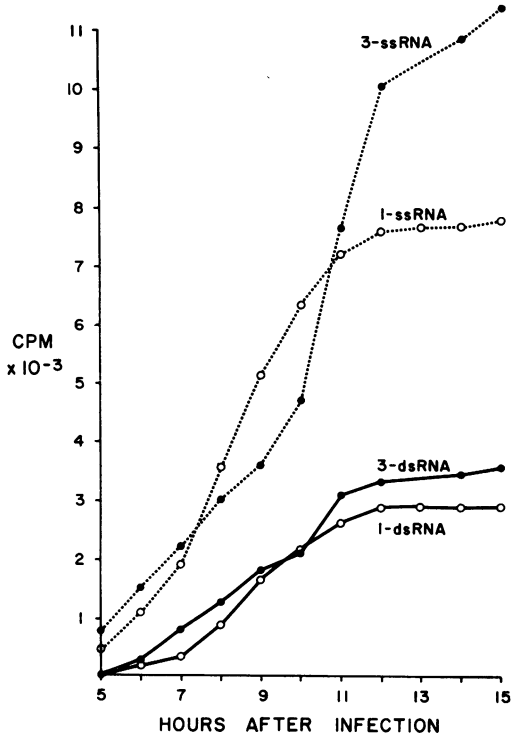

FIG. 5. *Kinetics of synthesis of ssRNA and dsRNA in cultures infected with either reovirus type 1 or type 3.*

to 100 counts/min; the area under each peak was then computed as a percentage of the total (Table 1).

Clearly, in cells infected with type 3 virus, the amounts of ssRNA-1, ssRNA-2, and ssRNA-3 made at any time are equal to one another, at least until 12 hr after infection. Some lysis starts to occur at approximately 14 hr after infection, and such analyses cannot be carried out past this time. Virus growth curves shown in Fig. 7 indicate that maturation commences no later than 5 hr and is virtually complete by 15 to 16 hr after infection. Thus, the results shown in Fig. 6 cover the major period of virus multiplication. In cells infected with type 1 virus, the amounts of the three types of ssRNA synthesized at various times are again in fixed ratio to each other, but the amounts are not equal; relatively much less ssRNA-3 is made in the type 1 than in the type 3 system.

Synthesis of ssRNA in BHK21 cells infected with type 1 reovirus. There was a possibility that the decreased amount of ssRNA-3 formed after infection of L cells with type 1 virus was specifically related to the use of L cells as host. Such a possibility was suggested by the observation of Oda and Joklik (13) that the pattern of synthesis of early and late mRNA induced by infection of Hela cells with vaccinia virus differs markedly from that in L cells. To look into this question, BHK21 cells were infected with type 1 virus and the ssRNA formed between 10 and 12 hr was labeled, extracted, and analyzed. The results (Fig. 8) clearly show that the relative amounts of ssRNA-1, ssRNA-2, and ssRNA-3 synthesized were similar to those formed in L cells under similar conditions.

DISCUSSION

Three conclusions may be drawn about virus-specific RNA synthesis in cells infected with type 1 reovirus.

(i) The dsRNA extracted from infected cells gives the same pattern as type 3 dsRNA on electrophoresis through a polyacrylamide gel column. Thus, the genome of type 1 virus is fragmented on extraction to give the classes dsRNA-1, dsRNA-2, and dsRNA-3 with the same molecular weights and in the same molar ratio, 3:2:2, respectively, as found for type 3 virus (19).

(ii) The ssRNA obtained either from polyribosomes or directly from the infected cells can be fractionated into three classes by sedimentation on sucrose gradients. These classes correspond in molecular size to the three ssRNA fractions isolated by similar methods from cells infected with type 3 virus. Moreover, each ssRNA hybridized specifically with a corresponding double-stranded fragment isolated from the type 1 viral RNA genome. The situation in the type 1 system is similar to that in type 3-infected cells: ssRNA-1 is is copied uniquely from dsRNA-1, ssRNA-2 from dsRNA-2, and ssRNA-3 from dsRNA-3, the points of breakage in the viral genome representing full stops in its transcription.

(iii) ssRNA-1, ssRNA-2, and ssRNA-3 are made in the weight ratio of 2:2:1, respectively (Table 1), throughout the course of infection. By comparison, the quantities of the three mRNA classes synthesized in type 3-infected cells are almost equal to each other at any time during the infectious cycle, as Gomatos has also shown recently by use of much shorter periods (15 min) to label the type 3 virus-induced mRNA (6).

Thus, in neither of the two reovirus systems is there a class of mRNA that can yet be characterized as "early" or "late," as has been found, for example, in cells infected with vaccinia virus (13) and in bacteria infected with T-even phages (5) or phage λ (12). If the major viral processes in the infected cell requiring synthesis of new mRNA commenced at approximately the same

time, it might be difficult to distinguish an "early" mRNA by the methods used here. In fact, the time at which the earliest mRNA could be detected, some 5 hr after infection, does correspond rather closely to the time when protein essential for the synthesis of progeny dsRNA starts to be formed (18), when progeny RNA itself can first be detected, and when viral maturation com-

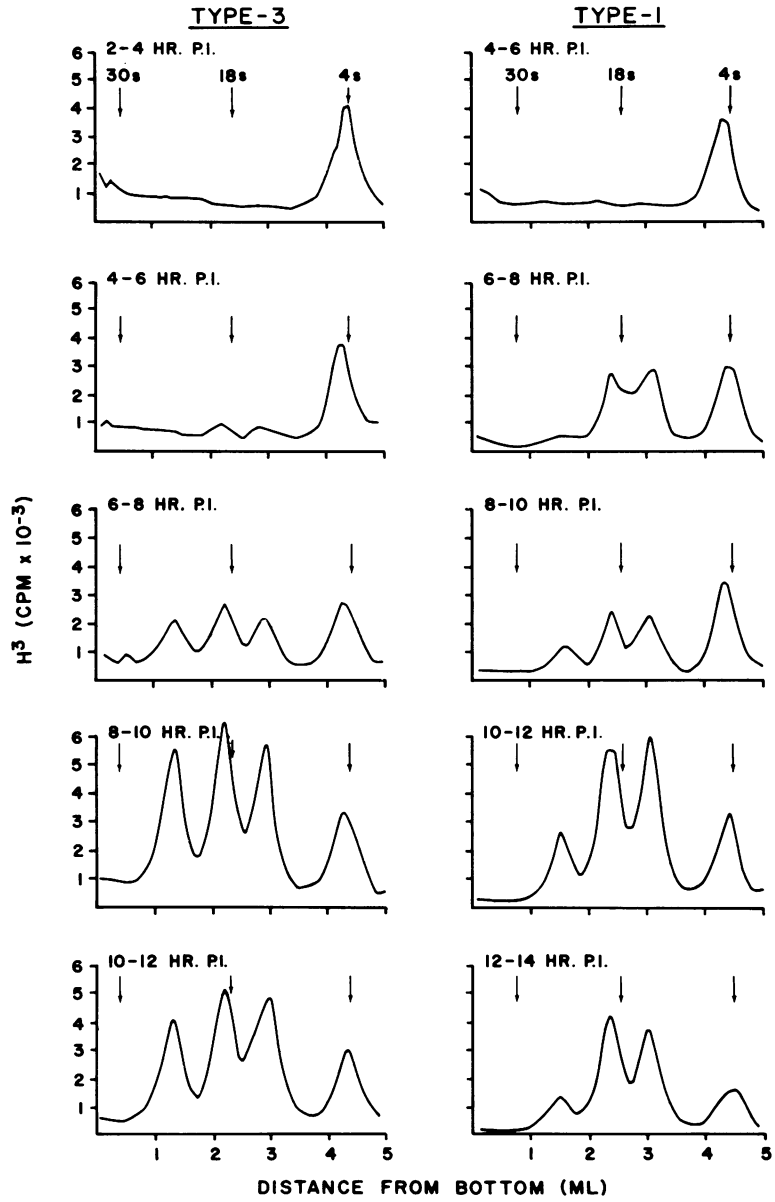

FIG. 6. *Sucrose gradient sedimentation analysis of ssRNA from type 3- and type 1-infected cells labeled at different times during the growth cycle. An L-cell culture was infected with type 3 reovirus. At time-zero, the culture was divided into five portions of 100 ml each. Cycloheximide (20 μg/ml) and additional actinomycin D (2 μg/ml) were added at 1.5, 3.5, 5.5, 7.5, and 9.5 hr postinfection. Thirty minutes later, in each case, ^{3}H-uridine (2 μc/ml) was added to the particular culture, and 2 hr later that culture was centrifuged and the RNA was extracted from the cells. The RNA was passed through a Sephadex G-25 column (5); a small portion of each sample was placed on a sucrose gradient together with 0.005 ml of ^{14}C-labeled L cell RNA as a sedimentation marker and was then centrifuged. The top of the gradient is to the right of the diagram. A second culture was infected with type 1 virus and treated similarly.*

mences under our experimental conditions. Furthermore, each class of dsRNA is comprised of more than one length of RNA (1, 17, 19), and, consequently, each corresponding class of ssRNA may contain more than one piece. If this is the case, the several lengths of mRNA within each of the three classes may well be synthesized at different relative rates at any given period and yet display the composite picture we have presented here. It is obvious that further analysis of this problem is going to depend on the development of methods for separating the individual classes of mRNA from each other.

TABLE 1. *Synthesis of three classes of ssRNA at different times after infection with type 1 and type 3 reovirus*

Period of labeling postinfection	Relative amounts of various classes of ssRNA (%)[a]					
	Type 3 virus			Type 1 virus		
	ssRNA-1	ssRNA-2	ssRNA-3	ssRNA-1	ssRNA-2	ssRNA-3
hr						
6–8	30	36	34	46	41	13
8–10	30	37	33	40	37	23
10–12	35	34	31	41	41	18
12–14	—	—	—	41	44	15
Average	32	36	33	42	41	17

[a] ^3H (counts per minute) in each fraction as percentage of total in the three fractions.

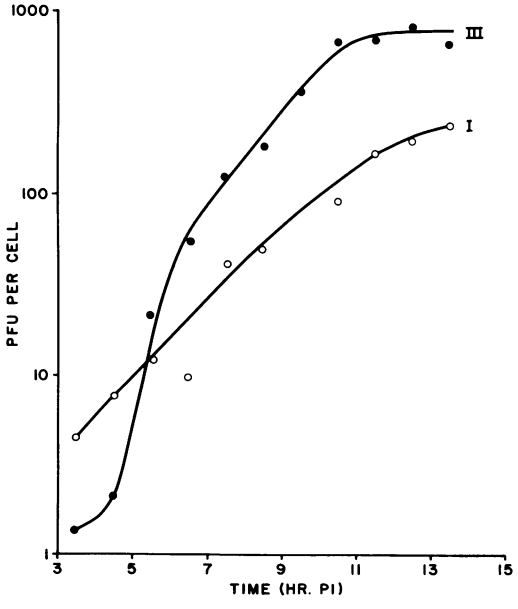

FIG. 7. *One-cycle growth curves of reovirus types 1 and 3 in L cells. The curves represent the total amount of virus in each of the cultures of Fig. 5. Samples taken at intervals from those cultures were frozen and thawed five times and assayed for plaque-forming units.*

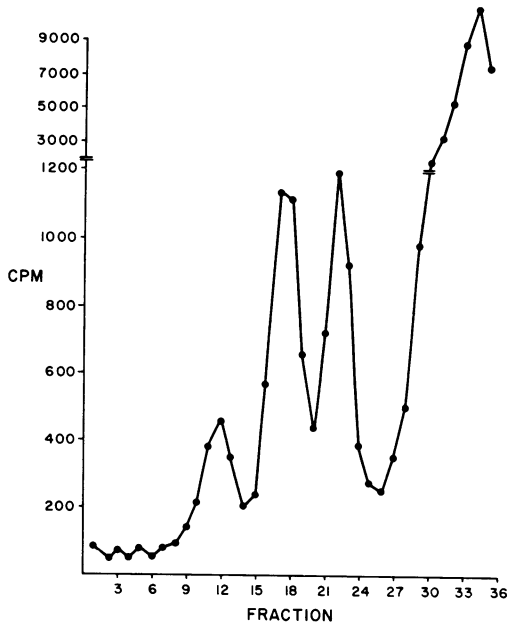

FIG. 8. *ssRNA from BHK21 cells infected with reovirus 1. Monolayer cultures of BHK cells were trypsinized and placed in suspension culture at a concentration of 8 × 10^5 cells/ml in the usual growth medium. Six hours later, the cells were sedimented by centrifugation and infected with type 1 reovirus. At 9.5 hr postinfection, cycloheximide (20 μg/ml) and additional actinomycin D (2 μg/ml) were added. ^3H-uridine (1 μc/ml) was added at 10 hr postinfection. The cells were harvested at 12 hr, and the RNA was extracted and analyzed by sucrose gradient centrifugation. The top of the gradient is to the right.*

Basically, two types of model can be postulated for transcription of the reovirus genome. According to the first model, the various segments of viral RNA would be arrayed in linear fashion, end to end, and the genome would be copied continuously from one end to the other in the infected cell. In this event, the amounts of the three classes of mRNA formed should be in the same ratio as the amounts of the dsRNA classes. That is, the relative amounts of dsRNA-1:dsRNA-2:dsRNA-3 are approximately 1:1.3:2 (19), and these should also be the relative amounts of ssRNA-1:ssRNA-2:ssRNA-3. The results in Table 1 are in marked disagreement with such a simple model of transcription. In both the type 1 and type 3 virus systems, the two smaller classes of viral genome are more often copied than this

model would predict. In addition, some restriction is placed on transcription of the dsRNA-3 segments of the type 1 viral genome by comparison with the type 3 system. Both these pieces of evidence are in favor of the second type of model, which would postulate that each segment of genome is copied independently of any other during the infectious cycle. There is insufficient evidence as yet to specify any details of such a model, but it is clear that, with some restriction placed on the copying of the dsRNA-3 segments of type 1 virus, there is control being exerted in the reovirus system at the level of transcription. Actually, the amount of ssRNA-3 formed after type 1 virus infection seems to be about half that of ssRNA-1 and ssRNA-2. Since it has been postulated that there are two segments of dsRNA-3 in the viral genome (19), the question naturally arises whether transcription of both segments of the type 1 genome is partially suppressed or whether transcription of one is totally restricted and the other is not restricted at all. This is a point which is open to test, and experiments to this end are in progress.

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