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Nonpermissive Infection of L Cells by an Avian Reovirus: Restricted Transcription of the Viral Genome

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Avian reovirus multiplies in chicken embryo fibroblasts. Although the avian virus adsorbs to L cells and is uncoated therein, it does not multiply. In the nonpermissive infection of L cells with the avian reovirus only four of the genomic segments of the viral genome are transcribed, L_1, M_3, S_3, and S_4, and these are the same segments that have been designated previously as early functions in the permissive infection of L cells with type 3 reovirus. When L cells are co-infected with avian reovirus and type 3 virus all ten segments of the avian viral genome are transcribed, although there is no synthesis of avian viral double-stranded RNA. Type 3 reovirus multiplies almost normally in this mixed infection. The most likely explanation is that a cellular repressor blocks transcription of the six late segments of the avian viral genome and that this repressor is removed by the co-infection with type 3 virus. A second block prevents replication of the viral genome.

Replication of the double-stranded (ds) RNA genome of reovirus in vivo is an asymmetric process in that all 10 segments of the parental genome must first be transcribed to provide the templates for progeny dsRNA synthesis (9). Transcription is carried out by an RNA polymerase which is an integral part of the parental virion (1, 11) and which is activated by the uncoating process in the cell (2, 12). There are two stages in transcription of the parental genome (8, 17). Four segments called the early segments are first transcribed, and there is then a rapid transition to the transcription of all 10 segments. It has been difficult to establish any mechanism for the regulation, since all of the known classes of temperature-sensitive mutants of the virus go through this same transition under nonpermissive conditions (3, 5, 17). Cycloheximide added at the time of infection permits the transcription of only the four early segments into functional mRNA's (6, 20). However, beyond demonstrating that virus-specific protein synthesis is essential to turn on transcription of all the parental segments, cycloheximide inhibition has not been particularly helpful in further analysis of the system.

In carrying out some recent experiments with an avian strain of reovirus (16) we observed that it would adsorb to L cells and be uncoated, but there was no replication of the viral genome. Further analysis of this abortive infection indicated that only the four early segments of the avian viral genome were transcribed. When L cells were mixedly infected with avian reovirus and type 3 reovirus all 10 segments of the avian viral genome were transcribed, although the avian viral genome was not replicated. The results of this study are presented here.

MATERIALS AND METHODS

Cells and virus. Primary cultures of chicken embryo fibroblasts (CEF) were prepared from 9- to 10-day-old embryos and grown in minimum Eagle medium (MEM) supplemented with 10% tryptose phosphate broth and 10% fetal calf serum (FCS). When these cultures became confluent they were trypsinized and used for the preparation of secondary cultures which were used throughout in experiments with avian reovirus. L cells were grown in suspension in MEM containing 5% FCS.

The S1133 strain of avian reovirus was used (16, 19). A plaque was picked from an assay of the virus on CEF and grown up to a large stock by five serial passages in CEF. The R_2 strain of reovirus serotype 3 was propagated in L cells (15). Purification, preparation of labeled virus, and plaque assay for the avian and R_2 viruses have been described, as have the buffers, chemicals, and isotopes (15, 16).

Uncoating of avian reovirus in L cells. ^{3}H-labeled purified avian reovirions were adsorbed to L cells in suspension for 2 h at 4°C at a multiplicity of infection of 100 PFU/cell. Unadsorbed virions were removed by washing the cells with phosphate-buffered saline. After the final centrifugation the cells were resuspended in MEM containing 2% heat-inactivated FCS and divided into two. After 3 h of incubation at 37°C for one culture and 10 h for the other the cells were harvested by centrifugation, and cytoplasmic fractions were prepared (17). These frac-

tions were mixed with ^{14}C-labeled avian reovirus to act as a density marker and analyzed on CsCl equilibrium density gradients.

Preparation of ssRNA from virus-infected cells. Suspension cultures of L cells were infected at a multiplicity of infection of 100 PFU/cell with either R_2 virus or avian reovirus (titrated in CEF). Virus was adsorbed for 2 h at 4°C at a cell concentration of 5×10^6/ml, and the cells were then washed with phosphate-buffered saline and resuspended in MEM containing 2% heat-inactivated FCS and 0.5 μg of actinomycin D per ml. The final volume of culture was 200 ml containing 5×10^5 cells/ml. The cultures were incubated at 37°C. Virus-specific RNA was labeled by the addition of 5 μCi of [^3H]uridine per ml for intervals to be later specified. At the end of each labeling period the cells were centrifuged and washed with phosphate-buffered saline and the RNA was extracted with phenol and sodium dodecyl sulfate at 60°C (16). This RNA was then analyzed by sucrose gradient sedimentation or used in hybridization experiments. Single-stranded (ss) RNA from L cells co-infected with avian and type 3 reoviruses was separated from dsRNA by precipitation with 1 M NaCl at 4°C overnight and then used for hybridization.

Hybridization of ssRNA transcripts and polyacrylamide gel electrophoretic analysis. Hybridization was performed as previously described (17) by mixing ^3H-labeled ssRNA extracted from infected cells with excess ^{14}C-labeled, denatured avian reovirus dsRNA. The resulting hybrids in 0.3 M STES buffer (0.3 M NaCl, 1 mM EDTA 50 mM Tris-chloride [pH 7.4], and 0.5% sodium dodecyl sulfate) were treated with pancreatic RNase (1 μg/ml) at 37°C for 10 min before being applied to polyacrylamide gels for electrophoretic analysis (17). The purpose of the RNase digestion was to remove any partial hybrids between type 3 ssRNA and avian reovirus dsRNA.

Transcriptase activity in SVPs formed in vivo and analysis of transcripts. Avian reovirus was adsorbed to 3×10^8 L cells at 4°C for 2 h at a multiplicity of 100 PFU/cell (titration carried out on CEF cells). The virus-cell complexes were incubated at 37°C for 10 h in MEM containing 2% heat-inactivated FCS. A postnuclear cytoplasmic extract was prepared (17), diluted with 0.05 Tris-chloride, pH 8.0, and centrifuged in an SW27.1 Beckman rotor for 1 h at 25,000 rpm at 4°C. The resulting pellet which contained the parental subviral particles (SVPs) was resuspended in 0.4 ml of Tris-chloride, pH 8.0, subjected to sonic oscillation, and incubated for 2 h in a 4-ml transcriptase reaction mixture containing [^3H]UTP (17). The labeled ssRNA products of this reaction were isolated, annealed with excess ^{14}C-labeled, denatured avian reovirus dsRNA, and analyzed by polyacrylamide gel electrophoretic analysis (PAGE) (17).

Isolation of virus from mixedly infected cultures. Monolayers containing a total of approximately 5.3×10^8 CEF cells (10 plastic bottles, 75-cm^2 growth area/bottle) were co-infected with avian and R_2 virus at multiplicities of 100 PFU/cell for each virus. Adsorption was for 2 h at 20°C. The cells were washed twice with 10 ml of phosphate-buffered saline, and then 25 ml of phosphate-free MEM containing 2% heat-inactivated FCS and 5 μCi of $^{32}P_i$ per ml was added per culture bottle. After 20 h of incubation at 37°C the cells and medium were removed and centrifuged in an SW27 rotor for 90 min at 25,000 rpm. Virus was purified from the resulting pellet as described (15). Control cultures of CEF were infected with 100 PFU of avian or R_2 virus per cell separately and treated in the same way. Similarly, suspension cultures containing 10^8 L cells were co-infected with R_2 and avian viruses at multiplicities of 100 PFU/cell for each virus. Adsorption was for 2 h at 20°C. The cells were washed with PBS, resuspended in 200 ml of phosphate-free MEM containing 2% heat-inactivated FCS and 5 μCi of $^{32}P_i$ per ml, and incubated at 37°C for 20 h. Virus was then purified. Control cultures of L cells were infected with 100 PFU of avian or R_2 virus per cell and treated in the same way.

Isolation and analysis of dsRNA extracted from mixedly infected cultures. Monolayers of CEF or L cells on plastic dishes (60 mm) were co-infected with 100 PFU each of avian and R_2 virus per cell. Adsorption was for 2 h at 20°C. The cells were washed with PBS, and 5 ml of phosphate-free MEM containing 2% heat-inactivated FCS and 5 μCi of $^{32}P_i$ per ml was added per dish. After 20 h of incubation at 37°C the cells and medium were harvested and centrifuged in an SW27 rotor for 90 min at 25,000 rpm. From the pellet the RNA was extracted with phenol at 60°C in the presence of 0.5% sodium dodecyl sulfate and 0.05 M sodium acetate buffer, pH 5.3. NaCl was added to the extract to give a final concentration of 0.3 M, and the RNA was precipitated by adding 3 volumes of ethanol. The RNA precipitate was dissolved in electrophoresis buffer and analyzed on 5% polyacrylamide slab gels. Since cellular or viral ssRNA and tRNA did not interfere in this analysis of viral dsRNA, there was no need for their prior separation.

RESULTS

Adsorption of avian reoviruses to L cells. The rates of adsorption of avian and R_2 reoviruses to L cells were measured, and the results are shown in Fig. 1. Both viruses adsorbed at approximately the same rate and to the same final extent of 75% in 2 h.

Uncoating of avian reovirus in L cells. To find whether the avian reovirus was uncoated, a sample of virus labeled in its RNA with ^3H was adsorbed to L cells. After periods of 3 and 10 h at 37°C cytoplasmic extracts were prepared from the culture, mixed with some ^{14}C-labeled virus to act as a density marker, and centrifuged in CsCl density gradients. After 3 h most of the adsorbed virus appeared at the SVP buoyant density of 1.40 g/ml (2, 12, 17) (Fig. 2). By 10 h after infection the conversion of adsorbed virus to SVPs was essentially complete.

Transcription of the avian viral genome in L cells. During the course of the permissive

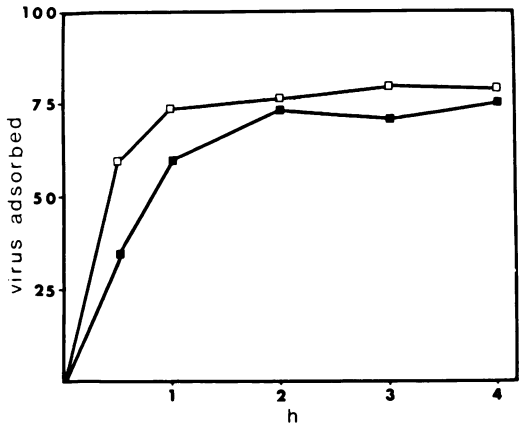

FIG. 1. *Adsorption of avian and R_2 reoviruses to L cells. Avian virus labeled with ^3H- and ^{14}C-labeled R_2 virus, each at a multiplicity of infection of 10 PFU/ cell, were added together in a volume of 0.2 ml to monolayers of L cells in 60-mm plastic dishes at 31°C. At intervals the unadsorbed virus was washed from duplicate plates with phosphate-buffered saline, its radioactivity was determined, and the percentage of each virus adsorbed was calculated. Symbols: ■, Avian reovirus; □, R_2 reovirus.*

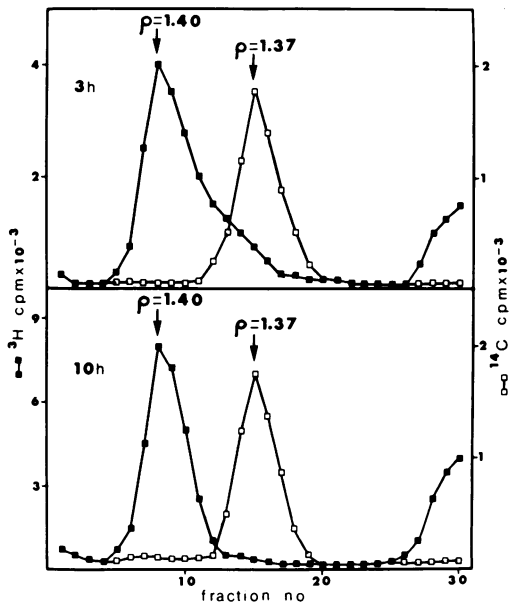

FIG. 2. *Isopycnic centrifugation in CsCl of subviral particles formed by uncoating of ^3H-labeled avian reovirus in L cells. Upper panel, SVP isolated 3 h postinfection; lower panel, SVP isolated 10 h postinfection. ^{14}C-labeled avian reovirus was used as a density marker and bands at 1.37 g/ml.*

infection of L cells with R_2 virus, transcription of the parental genome is carried out by the virion transcriptase which is activated in the uncoating process (2, 12). Since the avian virus was also uncoated by L cells, it would be expected that its genome would be transcribed by the avian viral transcriptase under these conditions. The following experiments were done to test this prediction.

(i) Sedimentation analysis of the avian viral transcripts. Three cultures of L cells were infected with avian reovirus and placed at 37°C. Actinomycin D was added to each culture. One culture was labeled between 2 to 6 h postinfection with [^3H]uridine; the other cultures were similarly labeled between 6 to 10 h and 10 to 14 h postinfection. At the end of each labeling period RNA was extracted from the cultures and analyzed by sedimentation on sucrose gradients, with the results shown in Fig. 3. During

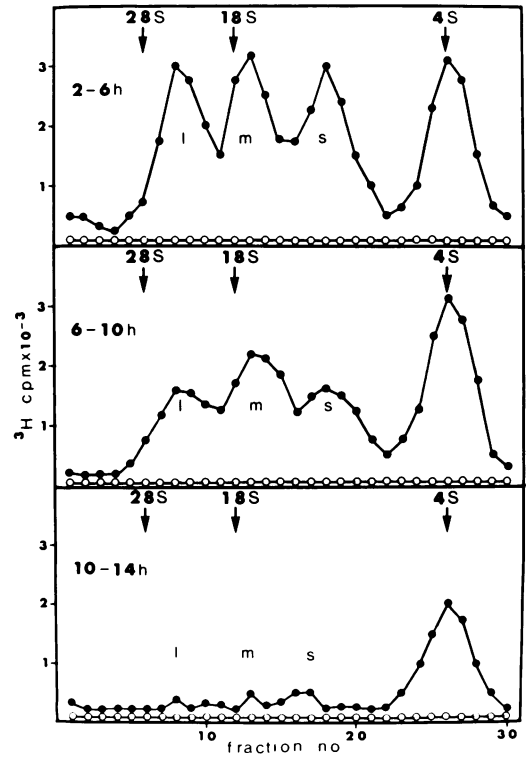

FIG. 3. *Sucrose gradient sedimentation analysis of ^3H-labeled RNA formed in L cells infected with avian reovirus. Upper panel, Infected cells labeled between 2 to 6 h postinfection with [^3H]uridine in presence of 0.5 μg of actinomycin D per ml; middle panel, labeled between 6 to 10 h postinfection; lower panel, labeled between 10 to 14 h postinfection. Arrows represent the positions of 28 and 18S ribosomal RNA and 4S RNA from the cells, and l, m, and s represent the three size classes of reoviral ssRNA. Each fraction was divided into two and assayed for trichloroacetic acid-precipitable ^3H (●) and trichloroacetic acid-precipitable ^3H (○) after treatment of each fraction with 10 μg of RNase per ml at 37°C for 1 h.*

the 2- to 6-h and 6- to 10-h labeling periods virus-specific ssRNA of the three size classes, l, m and s, was synthesized. The amounts of ssRNA formed decreased with time after infection, and during the 10- to 14-h period very little was synthesized. At no time was there any synthesis of viral dsRNA, represented by the open circles in Fig. 3. The avian viral genome is therefore transcribed but not replicated in this nonpermissive infection.

(ii) Analysis of the avian viral ssRNA by hybridization and PAGE. To determine the nature of the ssRNA formed in the previous experiment, samples of the RNA analyzed by gradient sedimentation (Fig. 3; 2- to 6-h and 6- to 10-h periods) were hybridized with ^{14}C-labeled dsRNA that had been extracted from purified avian reovirus. The resulting hybrids were analyzed by PAGE with the results shown in Fig. 4. Only four of the ten segments of the avian viral genome, L_1, M_3, S_3, and S_4, were transcribed during both time intervals. These are the segments we have previously defined as responsible for early viral functions in the permissive infection of L cells with type 3 virus (6, 8).

Thus avian virus cannot multiply in L cells, because transcription of its genome cannot proceed beyond the early stage. Relative frequencies of transcription are indicated by the $^3H/^{14}C$ ratios of the peaks in Fig. 4 (8, 17), and it is clear that the M_3 avian viral segment was transcribed with considerably higher frequency than the other three early segments. A similar observation was made for early transcription with type 3 reovirus (6, 8), but there is not yet

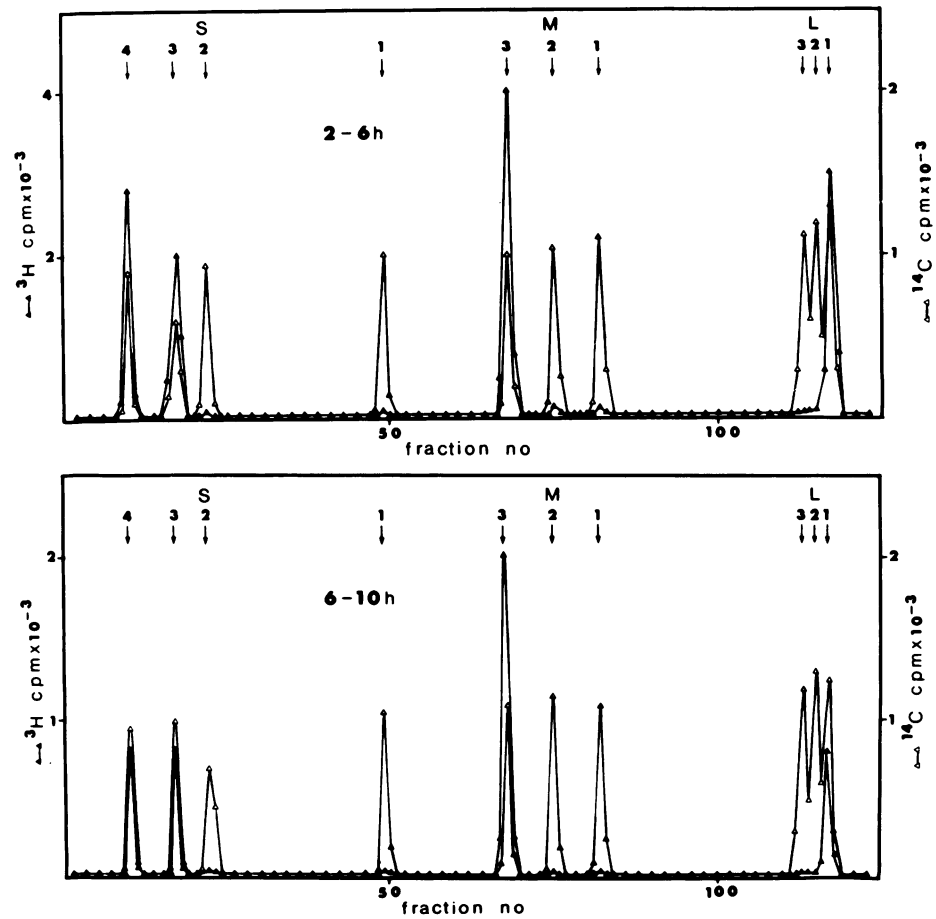

FIG. 4. *PAGE analysis of the hybrids formed between ^{14}C-labeled avian dsRNA and ^3H-labeled ssRNA synthesized in cells infected with avian reovirus. Upper panel, Infected cells were labeled between 2 to 6 h postinfection; lower panel, labeled between 6 to 10 h postinfection. Symbols: ▲, ^3H-labeled hybrids; △, ^{14}C-labeled dsRNA segments. L, M, and S are, respectively, the large, medium and small size classes of genomic segments.*

an explanation for the phenomenon.

In vitro transcription of SVPs isolated from L cells infected with avian reovirus. When SVPs are isolated from cells infected with type 3 reovirus and placed in an in vitro transcriptase system, all 10 genomic segments are found to be transcribed (12). It was therefore of interest to find whether SVPs isolated from L cells infected with avian reovirus would respond in the same way. Such viral particles were isolated 10 h after infection and placed in an in vitro transcriptase reaction mixture containing [^{3}H]UTP. The resulting ^{3}H-labeled transcripts were hybridized with ^{14}C-labeled dsRNA isolated from purified avian virus, and the hybirds were analyzed by PAGE. The results are shown in Fig. 5. All 10 segments were transcribed with frequencies inversely proportional to their molecular weights. Digestion of the SVP fraction with chymotrypsin prior to the reaction did not enhance its RNA transcriptase activity, suggesting that all the particles had been functionally uncoated in the cells. Similar results have been found with cores obtained by chymotrypsin digestion of purified reovirus and explained as the unregulated transcription of the viral genome (14, 17). Thus the restriction exerted in vivo on transcription of the avian viral genome was removed during isolation of the SVPs.

Analysis of virus and dsRNA formed in CEF or L cells during mixed infection with avian R_2 reoviruses. Since R_2 virus can grow in L cells but avian reovirus cannot, we wondered whether in a mixed infection of L cells with the two viruses the growth of R_2 virus might remove the restriction and permit the avian virus to multiply. CEF were therefore co-infected with a mixture of avian and R_2 viruses, or infected with each virus separately, and the cultures were labeled with $^{32}P_i$ as described in Materials and Methods. Similar experiments were done with L cells. Twenty hours later the virus was purified from the various cultures, and the number of virus particles in each preparation was determined with the results shown in Table 1. There was no growth of virus in

TABLE 1. *Virus yields from CEF and L cells infected with avian and R_2 reovirus*

Cells[a]	Infecting virus[b]	Virus yield particles/cell (× 10^4)[c]
CEF	Avian + R_2	2.8[d]
	Avian	16
	R_2	0[d]
L	Avian + R_2	1.5[e]
	Avian	0[e]
	R_2	7.6

[a] CEF, 5.3 × 10^8 cells were infected; L cells, 10^8 cells were infected. See Materials and Methods.

[b] The multiplicity of infection was 100 PFU/cell for each virus.

[c] In each case the virus was purified, and the total yield of viral particles in the purified preparation was calculated from the absorbancy at 260 nm. Assuming no loss of virus was incurred during purification, the yields of particles per cell were calculated from these results.

[d] No R_2 virus was detected by plaque titration of the cell lysate on L cell monolayers.

[e] No avian reovirus was detected by plaque titration of the cell lysate on CEF monolayers.

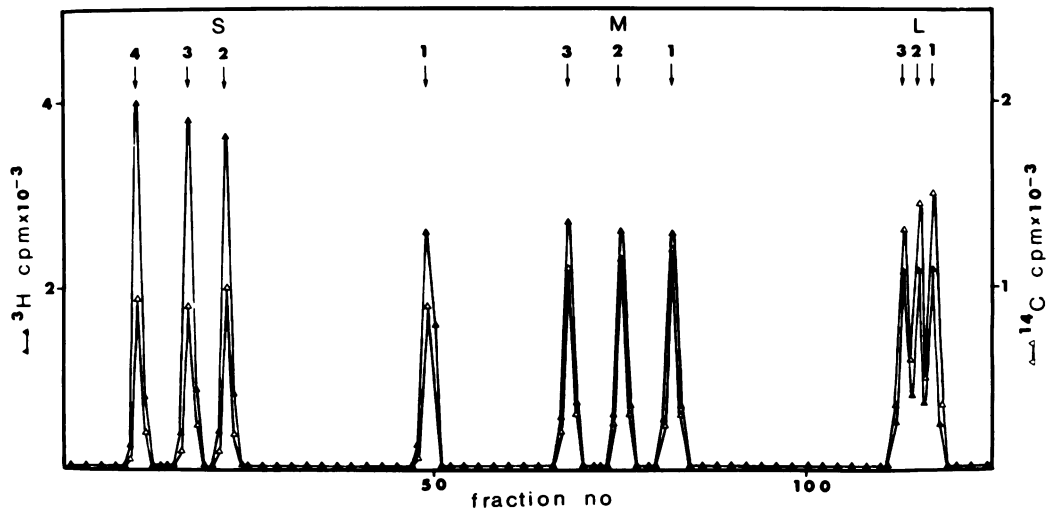

FIG. 5. *PAGE analysis of the hybrids formed between ^{14}C-labeled avian viral dsRNA and ^{3}H-labeled ssRNA formed in vitro with SVPs. The SVPs were isolated 10 h after infection of L cells with avian reovirus. Symbols: ▲, ^{3}H-labeled ssRNA hybridized with dsRNA; △, ^{14}C-labeled dsRNA segments.*

avian cells infected with R_2 virus or in L cells infected with avian virus. The viral yields from the mixedly infected cultures were approximately 20% of those from cultures infected with the homologous virus alone. Thus the yield of homologous virus was somewhat reduced in these mixed infections, even though in neither case was there any growth of the heterologous virus.

To determine whether the virions obtained from the mixed infections were genomically the same as the parental infecting virions, dsRNA was extracted from the purified preparations and analyzed by PAGE on slab gels. For controls, dsRNA from R_2 virus and from avian virus was run in separate wells. As shown elsewhere there is no difficulty in distinguishing the segments of avian virus dsRNA from those of R_2 virus (16). The virus obtained from the mixed yield in L cells was exclusively R_2, whereas that from CEF was exclusively avian.

Although heterologous virus was not formed in detectable amounts in the mixed infections, there was a possibility that the heterologous genomes might be replicated without being encapsidated. Therefore a mixed-infection experiment similar to that just described was done. The infected cells were labeled with $^{32}P_i$, but in this experiment the total RNA of the cells was extracted at 20 h postinfection and analyzed by PAGE as described in Materials and Methods. If heterologous genomic segments had been present to the extent of 2% of the corresponding segments of homologous virus, they could have been detected in the autoradiograms of the gels. There was no detectable replication of the heterologous viral genomes in the mixed infections of CEF or L cells.

Transcription of the genome of avian reovirus in cells mixedly infected with avian and R_2 reoviruses. It is well known that in L cells infected with R_2 virus all segments of the genome must be transcribed prior to replication of dsRNA (9). Although the genome of the avian virus did not replicate in a mixed infection of L cells with R_2 virus, it was still possible that the growth of R_2 virus might remove the block in transcription of the avian genome and permit all its segments to be transcribed. This turned out to be the case as the following experiments show.

To demonstrate transcription of the avian viral genome in cells where R_2 virus is also multiplying requires that transcripts of the avian genome will hybridize specifically with avian dsRNA in the presence of excess R_2 viral ssRNA transcripts. A control experiment was therefore done to find the degree of cross homology between the two viral genomes. Transcripts of the R_2 viral genome were made in vitro using R_2 viral cores and [^{3}H]UTP in the transcriptase reaction mixture (17). These transcripts were hybridized to both ^{14}C-labeled R_2 viral dsRNA and ^{14}C-labeled avian viral dsRNA, and the resulting hybrids were treated with pancreatic RNase to determine the degree of homology. The hybrids between R_2 transcripts and dsRNA were stable to RNase, but the hybrids between R_2 transcripts and avian dsRNA were almost completely digested (Table 2). Thus, there was virtually no homology between the R_2 and avian viral genomes.

Cells were then mixedly infected with R_2 and avian reoviruses and labeled with [^{3}H]uridine between 2 and 10 h postinfection. The ssRNA was isolated and hybridized with denatured avian viral dsRNA, and the hybrids were analyzed by PAGE. The results show that in the mixed infection there was no longer a control on transcription of the avian viral genome, and all 10 of its segments were transcribed (Fig. 6; Table 3).

DISCUSSION

Sufficient information has accumulated on the transcription of the reoviral genome that a plausible hypothesis can be advanced to explain its regulation. We have found here that avian reovirus adsorbs to L cells and is uncoated, but the viral genome cannot be transcribed beyond the four early segments. When SVPs were isolated from these cells, however, and placed in

TABLE 2. *Hybridization between ^{3}H-labeled transcripts synthesized in vitro with R_2 reovirus cores and ^{14}C-labeled dsRNA of avian and R_2 reoviruses*[a]

^{3}H-labeled R_2 transcripts (μg/ml)	^{14}C-labeled avian dsRNA (μg/ml)	^{14}C-labeled R_2 dsRNA (μg/ml)	Resistance to RNase (%)[b]	
			^{3}H	^{14}C
5	100		2	76
0	100			85
5		100	82	78
0		100		86
5	0	0	1	

[a] The total amount of RNA present in each hybridization mixture was adjusted to 200 μg/ml by the addition of unlabeled L cell RNA.

[b] Portions of the hybridization mixtures were incubated with RNase (5 μg/ml) in 0.3 M STES at 37°C for 30 min. RNase-treated samples and untreated controls were precipitated with 5% trichloroacetic acid in the presence of 50 μg of yeast RNA as a carrier, the precipitates were collected on membrane filters (Millipore Corp.), and their radioactivity was determined.

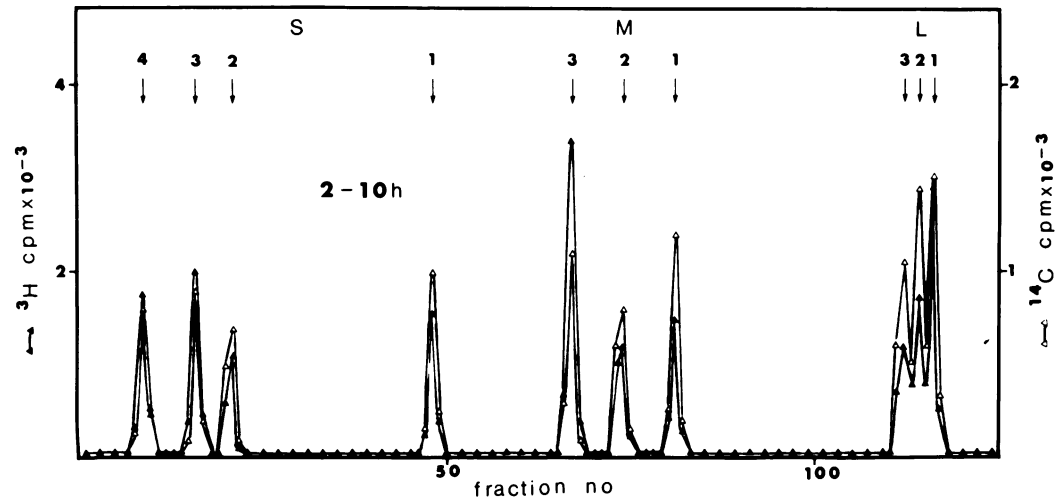

FIG. 6. *PAGE analysis of the hybrids formed between ^{14}C-labeled dsRNA of avian reovirus and ssRNA synthesized in L cells co-infected with avian and type 3 reoviruses. The cells were labeled between 2 and 10 h postinfection with [^{3}H]uridine. Symbols: ▲, ^{3}H-labeled ssRNA hybridized with avian viral dsRNA; △, ^{14}C-labeled avian dsRNA.*

an in vitro reaction system, all 10 of the genomic segments were transcribed. This situation is very similar to that found in the infection of L cells with type 3 reovirus in the presence of cycloheximide. Only four segments of the viral genome were transcribed in the inhibited cells (6, 8), but when the SVPs were isolated all 10 segments could be transcribed in vitro (12). In both these cases the restriction placed on transcription of the viral genome in the infected cells was removed by isolation of the SVPs. This in vivo restriction on transcription of the avian viral genome in L cells could also be removed by co-infecting the cells with type 3 virus, as has been shown in this paper. One further piece of information is pertinent. Shatkin and LaFiandra have shown that under controlled conditions type 3 reovirions can be partially uncoated with chymotrypsin in vitro and still retain most of their infectivity (10). When these partially uncoated virions are placed in an in vitro system all 10 genomic segments are transcribed. When they are used to infect L cells in the presence of cycloheximide, only the early segments are transcribed. These results together strongly suggest that a cellular function is involved in regulating transcription of the reoviral genome.

The simplest way of explaining these various observations is to suppose that a cellular component controls transcription of the reoviral genome and that this component is a repressor that regulates the action of the virion transcriptase. Such a mechanism would explain the loss of control on transcription when SVPs are isolated from cyclohexmide-treated cells or when avian viral SVPs are removed from L cells. We would further have to suppose that one of the early viral gene products can recognize the repressor and remove it in a permissive infection. This cannot happen in cycloheximide-treated cells, however, because no early viral proteins are synthesized. It would be presumed not to happen in L cells infected with the avian reovirus, because the early gene product(s) of the avian virus does not recognize the cellular repressor. Lack of derepression could also result if the early mRNA's of the avian genome were not translated in L cells, but this is unlikely, since we have obtained mutants of the avian virus that will grow in L cells (unpublished observa-

TABLE 3. *Relative frequencies of transcription of the genomic segments of avian reovirus in L cells co-infected with avian and R_2 reoviruses*

Genomic segment	^{3}H/^{14}C in the hybrids[a]
L_1	6.3
L_2	4.2
L_3	4.0
M_1	4.5
M_2	5.4
M_3	10.0
S_1	5.1
S_2	4.8
S_3	8.0
S_4	7.1

[a] ^{3}H/^{14}C ratios were determined from the profiles of Fig. 6 and normalized to a value of 10 for segment M_3 (8). These ratios give the relative frequencies of transcription of the avian genomic segments.

tions). Complete transcription of the avian viral genome can occur in L cells, on the other hand, if the cells are co-infected with type 3 reovirus. The repressor can be recognized and removed by the early gene product(s) of type 3 reovirus if not by that of the avian virus.

One alternative explanation is that during permissive infection of L cells an early viral gene product derepresses a cellular gene, and the resulting cellular component is involved in late transcription of the viral genome as a positive effect, for example, as a component of the transcriptase. In the nonpermissive infection with avian virus derepression of the cellular gene would be presumed not to occur because of lack of recognition by the avian viral gene product(s). This alternative would appear to be less likely, since it would not explain the unrestricted in vitro transcription found with avian SVPs isolated from L cells. Whatever may be the precise explanation it seems almost essential to invoke the participation of a cellular component in regulating transcription of the reoviral genome. Reovirus is thus one of a growing number of mammalian viruses that are thought to utilize specific cellular functions in their replication (4, 7, 18).

If a cellular repressor is involved in the reovirus system as suggested above, there is no indication as to what might be its nature or mechanism of action. The latter would pose a particularly difficult question, since the parental genome of reovirus never becomes free in the cell but is transcribed within a subviral structure which contains the polymerase (2, 12). Perhaps a repressor might act by altering the conformation of the polymerase, but the proof of any such mechanism will probably have to await the isolation of the hypothetical repressor.

ACKNOWLEDGMENTS

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