THE SYNTHESIS AND TURNOVER OF VIRUS-SPECIFIC POLYADENYLATED RNA IN POLYOMA-INFECTED CELLS

R. B. Rutherford and J. D. Hare

Department of Microbiology
University of Rochester School of Medicine and Dentistry
Rochester, New York 14642

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Summary

Mouse embryo cells infected with the 3049 strain of polyoma virus contain several fold more virus-specific, polyadenylated RNA beginning between 4 and 8 hours after the onset of viral DNA synthesis than do cells infected with wild-type virus (lpS). Following infection with either virus strain, there is an identical small but significant enhancement of the level of total polyadenylated RNA measured by binding of ^{125}I-labeled RNA to poly(dT)cellulose. The polyadenylation of "early" virus-specific RNA is inhibited 85-90% by cordycepin resulting in an "early" RNA preparation which competes fully with polyadenylated "early" virus-specific RNA in the ternary complex assay. Utilizing the non-polyadenylated "early" RNA, competition hybridization demonstrated that approximately 78% of the enlarged pool of "late" 3049 polyadenylated RNA and 72% of the "late" lpS pool consisted of sequences unique to the "late" period. No significant difference in the rate of decay of 3049 and lpS-specific, "late" polyadenylated RNA following actinomycin D block was found. Infection by either strain of polyoma virus did not alter the rate of decay of total polyadenylated RNA.

Recently we reported (1) that mouse cells infected with the 3049 strain of polyoma virus contained twice as much nuclear and polyribosomal, virus-specific, polyadenylated RNA as cells infected with a wild-type virus (lpS). The increased quantity of virus-specific RNA was detectable only following the onset of DNA synthesis. These findings correlated with the previous demonstration that cells infected with the 3049 strain contained capsid protein in the cytoplasm (2) and 3-5 times more capsid antigen than lpS-infected cells when measured by an indirect radio-immunoassay (Tachovsky, unpublished results). These data demonstrate a temporal relationship between accumulations of virus-specific, polyadenylated RNA and at least one class of late gene products, the capsid proteins.

We now report the kinetics of the appearance of virus-specific, polyadenylated RNA, as well as the turnover of virus-specific and host cell,

polyadenylated RNA. Furthermore, an analysis of the composition of the virus-specific, late RNA is presented.

Materials and Methods

Culture conditions: Infected secondary mouse embryo fibroblasts were prepared as described (1). Infected cells utilized for the production of nonpolyadenylated, virus-specific "early" RNA were refed with medium containing 15 µg/ml 3-deoxyadenosine (cordycepin, Calbiochem) and harvested at 24 hours after infection. Infected cells utilized in the experiments measuring decay of polyadenylated RNA were treated with actinomycin D at a final concentration of 10 µg/ml at 24 hours following infection and the RNA extracted at 4-hour intervals.

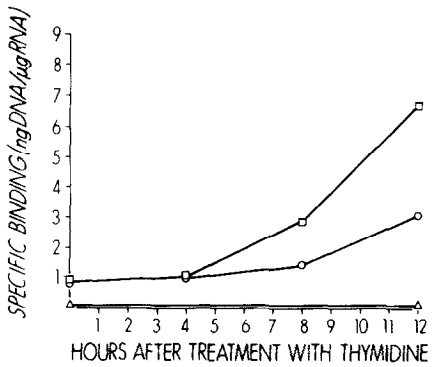
Infected-cell RNA: RNA was prepared from whole cells lysed in sodium dodecyl sulfate (SDS). The cells were first trypsinized, washed twice in cold, serumless medium and resuspended in 40 volumes of 0.01 M sodium acetate pH 5.1 made 0.5% with respect to SDS. The lysates were then extracted with phenol-chloroform isoamyl alcohol (3), the nucleic acids precipitated with ethanol or 2 M LiCl, treated with DNase (Worthington; electrophoretically purified, RNase free) and pronase (self-digested), sedimented through CsCl and resuspended in 2 x SSC (1 x SSC = 0.15 M NaCl, 0.015 M Na citrate) or 0.1 M sodium acetate pH 5.1.

Virus DNA and ternary complex assay mixtures: Polyoma component 1 DNA was purified and the ternary complex assay, originally described by Weinberg, Ben-Ishai and Newbold (7), was performed as reported (1).

Oligo(dT)cellulose assay: Binding of total cellular RNA extracted from virus and mock infected cells to oligo(dT)cellulose (Collaborative Research) was performed as reported by Singer and Penman (4) following labeling of the RNA with ^{125}I according to Commerford (5).

Results

The ternary complex assay utilized in these studies measures the binding of radio-labeled virus DNA to poly(U)treated glass fiber filters (6) through



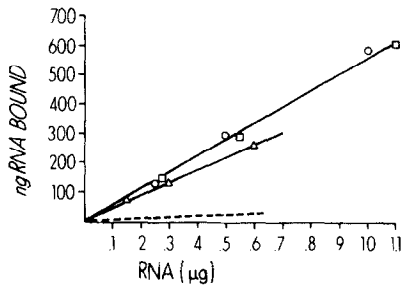


Fig. 1. Kinetics of accumulation of "late" virus-specific, polyadenylated RNA. Cultures were infected with virus and treated with FdU (15 μg/ml) for 24 hours. The FdU block was reversed at 24 hours after infection by treating with 20 μg/ml thymidine and the cells subsequently harvested at 4-hour intervals. Virus-specific, polyadenylated RNA was measured by the ternary complex assay. □—□ 3049 RNA; ○-○ lpS RNA; △-△ mock-infection RNA.

Fig. 2. Binding of "late" whole cell RNA, labeled in vitro with ^{125}I, to oligo(dT) cellulose. Infected cells were harvested after 24 hours' treatment with 15 μg/ml FdU followed by 12 hours' treatment with thymidine (20 μg/ml). ^{125}I-RNA was suspended in 0.4 M NaCl, 0.01 M Tris pH 7.4 made 0.5% with SDS incubated at room temperature with 150 μl of oligo(dT) cellulose for 5 minutes in presence of 500 μg yeast t-RNA. The cellulose was washed 3 times, dried and counted by gamma scintillation spectrometry. 3049-infected RNA □-□ ; lpS-infected RNA ○-○ ; mock-infection RNA △-△ ; average of 3049, lpS and mock-infection RNA pre-hybridized with 50 μg/ml poly (U), - - - - .

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the terminal poly(A) sequences on virus-specific RNA. The amount of DNA bound is directly proportional to the amount of RNA added to the reaction mixtures (1,7). Specific binding is defined as nanograms of virus DNA bound per microgram of infected cell RNA (1).

The accumulation of virus-specific, polyadenylated RNA was measured in infected cells harvested at 4-hour intervals following reversal of an FdU block of DNA synthesis. As shown in Figure 1, no difference in the quantities of virus-specific RNA in 3049 or lpS-infected cells was detected prior to 4 hours after reversal of the FdU block. By 8 hours, the level of virus-specific RNA in 3049-infected cells was significantly higher than in those infected by

lpS and by 12 hours the difference was greater than 2 fold.

This increased quantity of virus-specific, polyadenylated RNA following the onset of viral DNA synthesis could result from a 3049 virus-induced increase in the total amount of polyadenylated RNA. Total polyadenylated RNA was then measured by the binding of ^{125}I-labeled whole cell RNA to oligo(dT)-cellulose. As shown in Figure 2, there was no significant difference in the levels of polyadenylated RNA late in the lytic cycle of lpS and 3049-infected cells. The 3049-infected cell RNA measured in these experiments contained approximately twice as much virus-specific, polyadenylated RNA as did the lpS-infected cell RNA (data not shown). There was, however, a significantly greater ($p<0.005$) quantity of total, polyadenylated RNA in cells infected with both virus strains than in mock-infected cells harvested late in the lytic cycle. These data suggest that the increased levels of 3049 polyadenylated RNA are not secondary to a general increase in the total amount of polyadenylated RNA restricted to cells infected with 3049 virus.

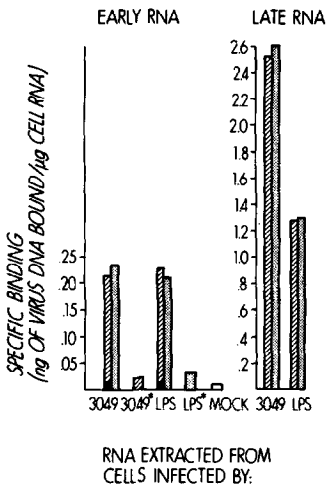
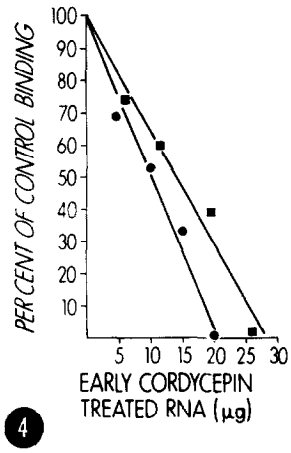


Fig. 3. The effect of cordycepin treatment on virus-specific RNA that binds to poly (U) filters in the ternary complex assay. Infected cells were treated with FdU (15 μg/ml) and cordycepin* (50 μg/ml) for 24 hours. Controls consisted of mock-infected cells as well as infected cells not treated with cordycepin and harvested at 24 hours and those harvested 12 hours following reversal of the FdU block at 24 hours after infection. Data have been corrected by subtracting the amount of DNA binding to filters incubated in the absence of RNA. This background binding never exceeded 1% of the input DNA. Cross hatched, 3049 DNA; stippled, lpS DNA; open, mock; solid, poly(U) plus reaction mixture.



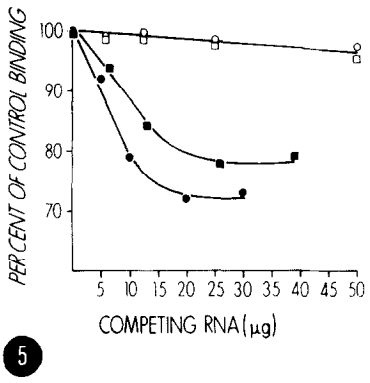


Fig. 4. Competition hybridization between "early" virus-specific RNA and "early" RNA from cordycepin-treated, infected cells. "Early" lpS and 3049-infected cell RNA (1.05 and 1.1 μg per assay, respectively) was incubated in 2 x SSC at 65°C for 10 hours (total volume = 0.2 ml) with ^{125}I-DNA (0.21 μg/assay) in the presence of homologous "early" RNA from cordycepin-treated cells. Data have been corrected by subtracting the amount of DNA bound by the homologous "early" cordycepin-treated RNA: 0.3 ng/μg for lpS and 0.2 ng/μg for 3049. ■-■ 3049 RNA; ●-● lpS RNA.

Fig. 5. Competition hybridization of "early" lpS, 3049 and mock-infected, cordycepin-treated, whole cell RNA with virus-specific, "late" whole cell RNA. 0.45 μg 3049 and 0.5 μg lpS RNA per assay were reacted with 0.2 μg of homologous DNA in the presence of increasing concentrations of "early" cordycepin-treated RNA. Percent competition by mock-infected "early", cordycepin-treated RNA was calculated after correction for the amount of DNA bound to poly (U) filters by this reagent: 2% for lpS and 2.6% for 3049.

■-■ 3049 ternary complex + 3049 "early" RNA (cordycepin)
●-● lpS ternary complex + lpS "early" RNA (cordycepin)
□-□ 3049 ternary complex + mock-infected RNA (cordycepin)
○-○ lpS ternary complex + mock-infected rNA (cordycepin)

Modifications of the ternary complex assay were used to measure the relative content of "early" and "late" virus-specific RNA 12 hours after the onset of viral DNA synthesis in 3049- and lpS-infected cells. For such competition experiments, the competing virus-specific RNA species must be free of the poly(A) moiety. It has been demonstrated that the polyadenylation of HeLa cell RNA can be inhibited 80% by cordycepin (4). The data depicted in Figure 3 demonstrate that treatment of lpS- and 3049-infected cells with cordycepin reduces the specific binding capacity of the "early" RNA 85 to 90%.

As shown in Figure 4, "early" RNA from cordycepin-treated cells competes fully with "early" RNA from untreated cells for the binding of ^{125}I-labeled, viral DNA. Whether cordycepin treatment also reduced the quantity of virus-specific RNA can not be discerned from these data.

Figure 5 presents competition curves demonstrating the reaction of "early" RNA (cordycepin) with "late" RNA from 3049- and lpS-infected cells. The "late" 3049 RNA analyzed in this experiment contained twice as much virus-specific, polyadenylated RNA than did "late" lpS RNA, as shown in Figure 3. Therefore, the 3049 RNA was diluted two-fold relative to the lpS RNA, allowing a direct comparison of the competition curves and facilitating the attainment of an excess of the competing RNA species. The data shown in Figure 5 demonstrate that 22% of the 3049 and 28% of the lpS-specific "late" sequences are similar to those present prior to the onset of DNA synthesis. These results indicate that a somewhat greater proportion of the enlarged pool of virus-specific polyadenylated "late" RNA from 3049-infected cells is composed of sequences unique to the "late" period.

Reciprocal competition experiments were not performed, since virus-specific RNA from cells treated with cordycepin after the onset of DNA synthesis would contain "early", polyadenylated sequences that would bind in ternary complex assay.

The relative stability of virus-specific, polyadenylated RNA following treatment of cells with actinomycin D was measured using the ternary complex assay and that of the total cell, polyadenylated RNA measured by the binding of ^{125}I-labeled RNA to oligo(dT)cellulose. These results are shown in Figure 6. On the assumption that this relationship was linear, a line of best fit was calculated and is also plotted. These data suggest that there is no significant difference in the rate of disappearance of the virus-specific, polyadenylated RNA in spite of the larger number of 3049-specific sequences. Furthermore, infection with either polyoma strain does not alter the rate of disappearance of total polyadenylated RNA when compared to that in mock-infected, control cells.

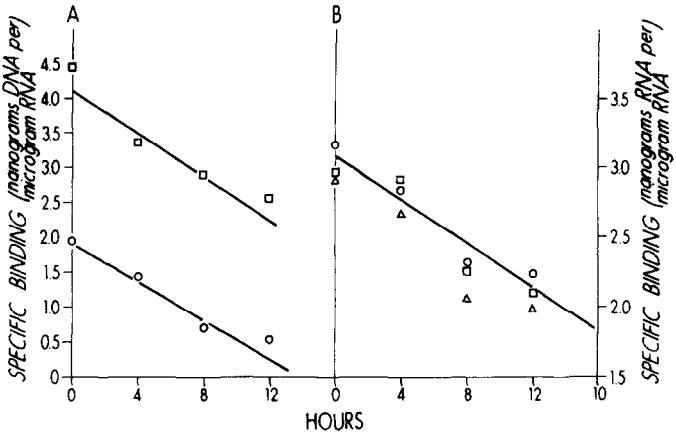


Fig. 6. Loss of whole-cell and virus-specific, polyadenylated RNA following actinomycin D block of RNA synthesis. Cells were infected with virus and treated for 24 hours with FdU, reversed with thymidine and treated with actinomycin D (10 μg/ml). Virus-specific, polyadenylated RNA was measured by the ternary complex assay (A). An aliquot of each RNA extract was labeled with ^{125}I and the content of total, polyadenylated RNA measured by binding to oligo(dT) cellulose (B). Binding to control cellulose was less than 10% of the binding to oligo(dT) cellulose. The data have been corrected for this nonspecific binding. 86% of the specific binding was sensitive to prehybridization with poly (U). ■–■ 3049 RNA; ●–● lpS RNA; ▲–▲ mock-infection RNA.

Discussion

The higher level of virus-specific, polyadenylated RNA in 3049-infected cells following the onset of virus DNA synthesis (Figure 1) does not appear to reflect simply an increase in the total polyadenylated RNA in cells infected by 3049 virus, as shown in Figure 2. While it is possible that a subset of the total polyadenylated RNA which includes the virus-specific material is elevated, these data support the hypothesis that the accumulation of 3049-specific polyadenylated RNA is the result of a virus function which operates only on virus-specific events.

The small but significant elevation of infected-cell, polyadenylated RNA above that detected in mock-infected cells is consistent with the marked increase of virus-specific RNA and the stimulation of several host cell functions late in the lytic cycle (8).

It has been shown that both "early" and "late" RNA sequences are tran-

scribed following the onset of polyoma (8,9) and SV40 (10) DNA synthesis. The majority of sequences unique to the late period function in the cytoplasm as mRNA for the synthesis of capsid protein. If a causal relationship exists between the enlarged pool of 3049 polyadenylated RNA present late in the lytic cycle and the increased quantities of capsid antigen, this RNA should contain a significant proportion of those sequences unique to the "late" period.

Experiments were carried out to measure the proportion of "early" RNA species in the "late" pool utilizing competing RNA from cells treated with cordycepin to inhibit the polyadenylation of this material (4). The results shown in Figure 3 demonstrate that cordycepin effectively inhibits (>85%) the polyadenylation of virus RNA as measured by the decrease in binding to poly(U)-charged filters. That the RNA extracted from infected cells following cordycepin treatment does indeed contain copies of all the virus-specific early transcripts is evidenced by the ability of this RNA to compete effectively with "early" RNA from untreated cells in the ternary complex assay, as shown in Figure 4.

The results of competition hybridization experiments shown in Figure 5 utilizing "early" RNA from cordycepin-treated, infected cells as the competing transcripts with "late" infected cell RNA demonstrate that in the pool of virus-specific, polyadenylated RNA molecules in both 3049- and lpS-infected cells, more than 70% of the sequences are unique to the late period. The presence of a high proportion of uniquely "late" sequences supports the hypothesis proposing a causal relationship between the enlarged pool of virus-specific, polyadenylated RNA and the accumulation of capsid antigen found late in the lytic cycle of 3049-infected cells.

A second interesting point may be raised from these data. There is a small reduction in the proportion of "early" sequences in 3049 as compared to lpS-infected cells which in turn indicates that the proportion of uniquely "late" 3049 sequences may be greater. This in turn suggests that the increased levels of total 3049 virus-specific sequences measured by the ternary complex assay may result from transcription of uniquely "late" regions of the genome

to a greater extent than "early" regions. Further experiments are required to determine whether this type of alteration in transcriptional regulation explains the phenotype of the 3049 virus strain.

The data from experiments designed to measure the turnover of virus-specific RNA supports the hypothesis that the higher levels of 3049-polyadenylated RNA result from enhanced production rather than increased stability of this material. Furthermore, infection with polyoma virus does not alter significantly the rate of decay of whole cell, polyadenylated RNA. These experiments indicate that this system may provide a means to study the process of degradation of a relatively limited population of mRNA molecules.

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