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Isolation and characterization of poly(A)-containing polyoma "early" and "late" messenger RNAs.

Leonard J. Rosenthal*

Department of Molecular Biology, University of Geneva, Geneva 4, Switzerland

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ABSTRACT

During late lytic infection of mouse kidney cell cultures polyoma 16S and 19S (late 19S RNA) were isolated by oligo(dT)-cellulose chromatography. Approximately 60-80% of total cytoplasmic polyoma RNA contained tracts of poly(A) which were retained by oligo(dT)-cellulose. Early in lytic infection when viral DNA synthesis and the production of capsid protein are blocked by the addition of 5-fluorodeoxyuridine, approximately 100% of polyoma "early" 19S RNA was quantitatively retained by oligo(dT)-cellulose indicating the presence of poly(A) tracts on most 19S mRNA molecules. In addition, 2 classes polyoma RNA, synthesized after the onset of cellular RNA synthesis under conditions where DNA synthesis is inhibited with 5-fluorodeoxyuridine, were found to contain tracts of poly(A). These species sedimenting at 16S and 19S in aqueous sucrose density gradients were also quantitatively retained by oligo (dT)-cellulose.

INTRODUCTION

During lytic infection of primary mouse kidney cell cultures with polyoma virus (Py), radioactive labeled Py specific RNA (Py RNA) can be detected by hybridization with Py DNA fixed on membrane filters (1,2). During the course of infection, a temporal sequence of events is observed beginning with the synthesis of small amounts of cytoplasmic, polyribosomal Py "early" 19S RNA. This is followed by the appearance of intranuclear Py-specific T antigen, induction of overall cellular RNA synthesis, and the replication of the host cell chromatin. The latter is paralleled by the synthesis of viral progeny DNA which can be isolated as a nucleohistone complex (3). Late in lytic infection when most cells participate in viral and cellular DNA synthesis, two classes of polyribosome-associated Py RNAs are found, a major component (80-90%) and a minor component (10-20%) with sedimentation coefficients of 16S

and 19S (late 19S RNA) respectively (4). In addition, during infection under conditions where DNA synthesis is inhibited with 5-fluorodeoxyuridine (FdU), two classes of cytoplasmic polyribosomal Py RNA sedimenting at 16S and 19S are observed (5). Similarly, early in SV40 lytic infection of monkey kidney cells the presence of viral specific 19S RNA has been reported, while late in lytic infection SV40-specific 16S and 19S RNAs are observed (6,7).

In order to understand the function(s) of the different classes of Py cytoplasmic mRNAs, it is essential to isolate these species in an intact form. Numerous reports have established that almost all mammalian cell and animal mRNAs contain polyadenylate [poly (A)] tracts varying in length from 150-250 nucleotides covalently linked to the 3' ends of the RNA molecules (8-11). Moreover, the presence of poly(A) tracts associated with SV40-specific RNAs from virally transformed and lytically infected cultures has been reported (12). In this publication, all species of Py cytoplasmic mRNAs synthesized both "early" and "late" were found to contain tracts of poly(A), thereby allowing the isolation of intact Py mRNAs.

MATERIALS AND METHODS

RNA Preparation: Confluent primary mouse kidney cell cultures (13) grown in 88 mm petri dishes containing 6-8 x 10^6 cells were infected (14) with plaque-purified wild-type polyoma viruses at a m.o.i. of 10-20. After adsorption the cultures were covered with serum-free reinforced Eagle's medium. In experiments where Py-induced DNA synthesis was to be inhibited, cultures were covered after the adsorption of the virus with medium containing 15 μg/ml of 5-fluorodeoxyuridine (FdU). Following infection cultures were labeled at either from 11-14 hr or 26-29 hr with medium containing 200-300 μCi/dish of [5-^{3}H]-uridine (New England Nuclear Corp., 25 Ci/mmol.). In experiments performed with FdU, the inhibitor was also present during the pulse with [^{3}H]-uridine. Following labeling the cultures were put on ice, washed with 5 ml ice-cold isotonic buffer (0.25 M sucrose, 10 mM triethanolamine (pH 7.4), 25 mM NaCl, 5 mM $MgCl_2$) and then lysed (15) by the

addition of 0.1 ml/dish of a 1% solution of Nonidet P40 (BDH Chemicals, Ltd.) in the same buffer. The lysate from 10-30 plates was collected by gently scraping the cells off the petri dishes into a pre-cooled Dounce Homogenizer. Gentle homogenization was carried out by hand for 15-30 sec until the preparation as examined microscopically contained 85-95% broken cells. The homogenate was transferred into Corex tubes and centrifuged at 1600 rpm for 5 min at 4°C in the Sorvall HB-4 rotor to remove the nuclei and large cellular particles. The supernatant fluid designated as the "cytoplasmic extract", was then diluted with 15 volumes of 0.01 M triethanolamine buffer (pH 7.4) (4°C) containing 50 mM NaCl, 6 mM EDTA, and 1.1% sodium dodecyl sulfate. An equal volume of a mixture of phenol-chloroform-isoamylalcohol in the ratio 50:50:1 (16) was added, the extraction mixture was shaken mechanically at room temperature for 20 min, and then centrifuged at 8500 rpm for 15 min in the Sorvall SS-34 rotor. The aqueous phase was reextracted with an equal volume of phenol-chloroform-isoamylalcohol mixture and recentrifuged. Following this the aqueous phase was removed and extracted with an equal volume of chloroform at room temperature for 10 min, centrifuged as described above, and the RNA precipitated by the addition of 1/40 volume of 5 M NaCl and 2.5 volumes of absolute ethanol.

Oligo(dT)-Cellulose Chromatography: Columns of oligo(dT)-cellulose-Type I (P-L Biochemicals, Inc.) were prepared according to Faust (17) in 10 mM tris (hydroxymethyl) aminomethane (Tris-HCl, pH 7.4) containing 0.5 M NaCl and 0.1% sodium dodecyl sulfate. RNA samples designated as "Load", diluted in the above buffer to a concentration of 160 µg/ml, were applied to a column containing 5 ml of oligo(dT)-cellulose. The column was then washed with four column volumes of 10 mM Tris-HCl (pH 7.4) containing 0.1 M NaCl, and the RNA which passed unbound after loading and washing was pooled and designated as "Flow-through". Finally, cytoplasmic and viral poly(A)-containing RNA, designated as "Oligo (dT)-retained" was eluted with two column volumes of 10 mM Tris-HCl (pH 7.4). Fractions of approximately 1 ml each were collected and 5-10 µl aliquots were

spotted on filter paper discs, precipitated with 5% trichloroacetic acid, and counted. The fractions containing poly(A)-mRNA radioactivity were pooled (3-4 ml) and concentrated 10 x under vacuum using collodion membranes.

Sedimentation in Sucrose Gradients:

Linear sucrose gradients (3.9 ml) (10-25%, w/v) were prepared in 10 mM Tris-HCl (pH 7.4) containing 0.05 M NaCl and 0.001 M Na_2 EDTA. RNA samples were diluted in 100 µl of the same buffer and layered over such gradients. Centrifugation was at 50,000 rpm for 2.5 hr at 10°C in a Spinco SW 56 rotor. Fractions were collected from the bottom of the tube into polyethylene tubes and aliquots of 10 µl were dried on filter-paper discs. These discs were washed at first in 5% trichloroacetic acid, then absolute ethanol, and the radioactivity was counted in toluene-based scintillation fluid. The remainder of each fraction was adjusted to a concentration of 4 X SSC (SSC: 0.15 M NaCl-0.015 M Na_3 citrate, pH 7.4) and hybridized as discussed below.

DNA-RNA Hybridization Technique:

DNA-RNA hybridization used to detect Py RNA in sucrose gradients was performed according to the procedure reported by Acheson *et al.* (18). Purified Py DNA I, converted into single strands, was fixed onto membrane filters (Schleicher and Schuell, B6) (19). Two 3.5 mm filters (minifilters) each containing 0.2 µg of Py DNA, and a blank filter were added to 100 µl of [^{3}H] RNA solution in 4 X SSC in a 300-µl capped polyethylene tube, and incubated at 65°C for 40-48 hr. The filters were washed in 4 X SSC, incubated with 20 µg/ml of pancreatic RNase (treated at 80°C for 15 min to inactivate possible traces of DNase) in 2 X SSC for 1 hr at 37°C, washed extensively in 4 X SSC, dried, and radioactivity counted in toluene based scintillation fluid. Py RNA is defined as the total radioactivity remaining bound to RNase-treated filters.

RESULTS

Isolation of Poly(A) - Containing Py "late" 16S and 19S mRNAs

It has been reported (4) that cytoplasmic polyribosomal Py mRNAs synthe-

sized late during normal lytic infection (26-29 hr) consisted of at least two classes of mRNA, a major and minor fraction with sedimentation coefficients in aqueous sucrose gradients of 16S and 19S respectively. In experiments shown in Fig. 1, polyoma-infected mouse kidney cell cultures were labeled with 200 μCi/dish of [^{3}H]-uridine for 3 hr between 26 and 29 hr after infection. At this time, virtually all cells contain intranuclear polyoma-specific T antigen and 70-80% of the cells synthesize both viral and cellular DNA (20). The cells were then lysed and cytoplasmic RNA was extracted by the procedure described in Materials and Methods.

Figure 1A represents the sedimentation profile of an aliquot of total RNA (Load); each fraction was then exhaustively hybridized with Py DNA to determine Py RNA. The results show that the bulk of total radioactivity sediments, as expected, mainly within the bands of 28S and 18S ribosomal RNA, and the remainder in a band sedimenting at 4 to 7S. The bulk of the radioactively hybridizable Py RNA sediments as a broad band, the peak exhibiting a sedimentation coefficient of about 16S.

The remainder of the total sample analyzed above was then passed over an oligo(dT)-cellulose column. Figure 1B shows the sedimentation profile of Oligo(dT)-retained total and Py RNA. The bulk of total radioactivity forms a polydisperse band sedimenting between 10-40S with a distinct band sedimenting at 16S. As revealed by hybridizations most of the Py mRNA (which accounts for 60% of total Py RNA in Load) sediments at 16S with a suggestion of a shoulder at 19S. The sedimentation pattern of Py RNA is in excellent agreement with that of Py RNA present in polyribosomes of polyoma infected mouse kidney cell cultures (4). It should be noted that Oligo(dT)-retained RNA from mock infected cultures showed a sedimentation profile shifted to higher sedimentation values (unpublished). The pronounced peak at 16S of total Oligo(dT)-retained RNA shown in Figure 1B was absent in the mock infected cells and is thus due to Py 16S RNA.

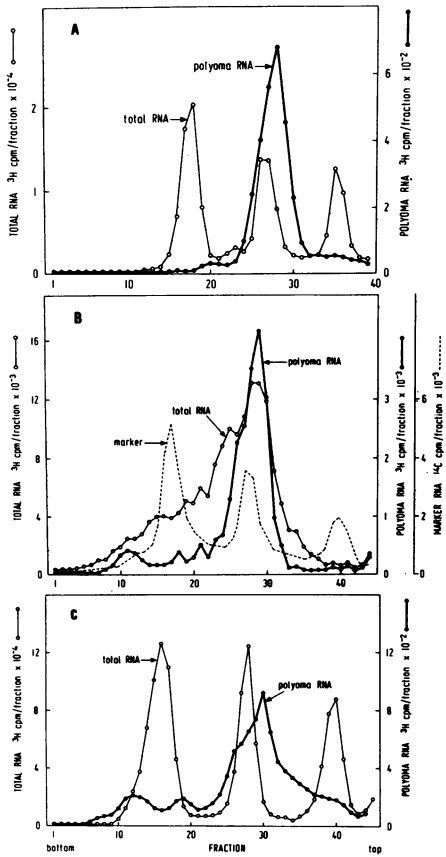

Figure 1

A) Sucrose gradient sedimentation analysis of total cytoplasmic RNA (Load) from polyoma-infected mouse kidney cells labeled with [^{3}H]-uridine (200 μCi/dish) between 26-29 hr after infection. About 8 μg [^{3}H] RNA (18,000 cpm/μg) was applied to a 10-25% sucrose gradient and centrifuged in a Spinco SW 56 rotor at 50,000 rpm for 2 hr and 15 min at 10°C. Polyoma-specific RNA determined by hybridization to Py DNA immobilized on membrane filters accounted for approximately 2.3% of the total cytoplasmic radioactivity. B) Sucrose gradient sedimentation analysis of Oligo(dT)-retained RNA obtained from total cytoplasmic RNA (Load) in Fig. 1A. Approximately 1.6 mg of Load RNA (18,000 cpm/μg) was applied to a 5 ml column of oligo(dT)-cellulose and the RNA retained accounting for about 8% of total was eluted according to procedures described in Materials and Methods. About 240,000 cpm of Oligo(dT)-retained RNA was mixed with about 2 μg of mouse cell RNA labeled with [^{14}C]-uridine (28,000 cpm/μg) and loaded onto a 10-25%sucrose gradient. Centrifugation was for 2.5 hr at 50,000 rpm in a Spinco SW 56 rotor. Fractions were collected and hybridized; Py-specific RNA was about 11% of total radioactivity retained on the column. C) Sucrose gradient sedimentation analysis of Flow-through RNA following passage of Load RNA in Fig. 1A over oligo(dT)-cellulose. About 60 μg of Flow-through RNA (1.1 x 10^6 cpm) was loaded onto a 10-25% sucrose gradient and centrifuged for 2.5 hr at 50,000 rpm in a Spinco SW 56 rotor at 10°C.

In Figure 1C the profile of the Flow-through RNA is analyzed under the same conditions as the RNA preparations in Figs. 1A and 1B. The total RNA profile is closely similar to Load (total cytoplasmic RNA) suggesting that the RNA remained essentially intact during oligo(dT)-cellulose chromatography and subsequent handling. This is confirmed in experiments in which intranuclear "giant" RNA retained on oligo(dT)-cellulose was examined under denaturing conditions in dimethyl sulfoxide gradients (Rosenthal et al., this volume). However, the profile of Py RNA, accounting for 40% of total Py RNA in Load, differed from that shown in Figs. 1A and 1B. In the Flow-through (Fig. 1C), though a peak at 16S was present, the bulk of Py RNA exhibited a rather polydisperse distribution with a trailing on the slower side. Upon subsequent repassage of the Flow-through over oligo(dT)-cellulose, retention of an additional 20% of Py RNA originally present in the Flow-through was retained by the column. In different experiments the relative amounts of radioactive hybridizable RNA in the Flow-through varied between 20-40%. This, considered together with the altered sedimentation profile in the Flow-through suggests that either a proportion of the Py-RNA lacks poly(A) or that the poly(A) tracts are too short to allow retention by oligo(dT)-cellulose (21-23). Due to the amount of oligo(dT)-cellulose present in the column for binding poly(A) tracts and considering the near quantitative recovery of early 19S mRNA (see below), it appears unlikely that the presence of late Py RNA in the Flow-through is a result of overloading.

Isolation of Poly(A) - Containing Py "Early" 19S mRNA

To obtain Py "early" 19S mRNA, mouse kidney cell cultures were infected in the presence of FdU and labeled between 11-14 hr after infection with [^{3}H]-uridine (+FdU). Cytoplasm was isolated, RNA was extracted, processed, and analyzed under the same conditions described above.

Figure 2A depicts the sedimentation profile in an aqueous sucrose density gradient of the Load. The bulk of total radioactivity is again found in 28S,

18S ribosomal RNA and 4-7S cellular RNA. Py RNA is seen as a band with a peak sedimenting at 19S as well as a fraction sedimenting more slowly extending into the 4-7S region. In all preparations examined the sedimentation pattern of Py RNA is similar and contained this slower sedimenting Py RNA though varying in relative amounts (5,24).

Figure 2B shows the sedimentation profile of the Oligo(dT)-retained RNA in an aqueous sucrose density gradient. The pattern of total radioactivity shows a broad sedimentation profile from 7 to > 40S and corresponds essentially to that of Oligo(dT)-retained RNA from mock-infected control cultures (unpublished). Compared with the profile of Oligo(dT)-retained RNA from cultures extracted late in lytic infection (Fig. 1B), this profile shows a marked shift to higher sedimentation values and an absence of the conspicuous peak of radioactivity mainly corresponding to 16S Py RNA. Following hybridizations the Py RNA is shown to exhibit a peak sedimenting at 19S as well as small amounts of hybridizable RNA sedimenting from 7 to 16S.

When the Flow-through RNA is analyzed (Fig. 2C), the pattern of total radioactivity is similar to that depicted in Fig. 1C. Little if any Py RNA can be detected in the 19S region although small amounts of slowly sedimenting Py RNA are observed. The results reported here show that Py "early" 19S mRNA is quantitatively retained by oligo(dT)-cellulose and suggest the presence of poly(A) tracts on most 19S mRNA molecules.

Isolation of two classes of poly(A)-containing Py RNA synthesized after the onset of cellular RNA synthesis under conditions where DNA synthesis is inhibited with FdU

There is asynchrony of polyoma induced DNA synthesis in individual cells. FdU (15 μg/ml) inhibits synthesis of cellular and viral DNA and of mouse chromosomal and Py capsid proteins. However, the early events of infection, i.e. synthesis of early 19S mRNA, T antigen, induction of cellular RNA synthesis, and the activation of the cellular DNA synthesizing apparatus occur as in normally infected parallel cultures. After infection for about 25 hr

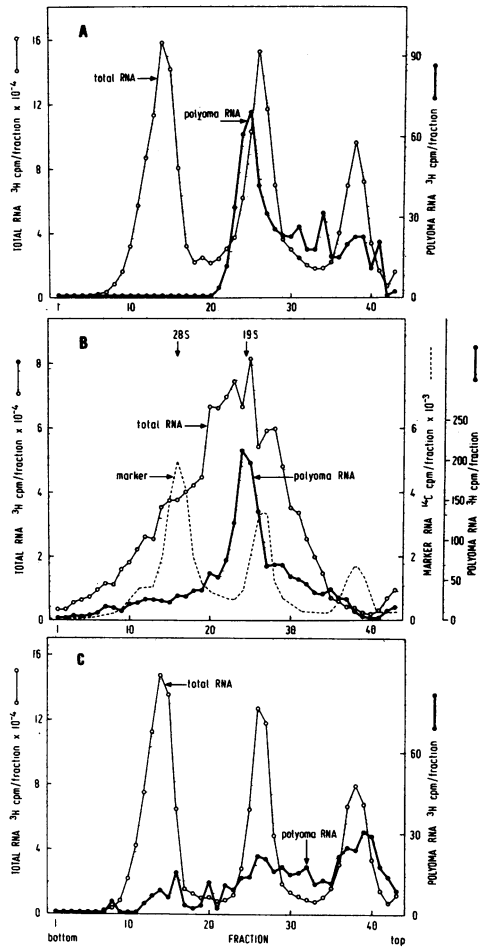

Figure 2

A) Sucrose gradient sedimentation analysis of total cytoplasmic RNA (Load from polyoma-infected mouse kidney cells labeled with [^{3}H]-uridine (300 μCi/dish between 11-14 hr after infection in the presence of FdU(15 μg/ml). About 140 μg of Load RNA (2 x 10^6 cpm) was applied to a 10-25% sucrose gradient and centrifuged at 44,000 rpm for 3 hr in a Spinco SW 56 rotor at 10°C. Polyoma-specific RNA determined by hybridizations was approximately 0.02-0.03% of total. B) Sucrose gradient sedimentation analysis of Oligo(dT)-retained RNA from total cytoplasmic RNA (Load) in Fig. 2A. Approximately 2.5 mg of Load RNA (14,000 cpm/μg) was applied to a 5 ml column of oligo(dT)-cellulose and approximately 11% of total radioactivity loaded onto the column was retained. About 20 μg of Oligo(dT)-retained RNA (3.6 x 10^6 cpm) was mixed with 2 μg (28,000 cpm/μg) of [^{14}C] marker RNA and loaded onto a 10-25% sucrose gradient. Centrifugation was 3 hr at 44,000 rpm in the Spinco SW 56 rotor at 10°C. C) Sucrose gradient sedimentation analysis of Flow-through RNA following passage of Load RNA in Fig. 1C over oligo(dT)-cellulose. About 180 μg of Flow-through RNA (2.5 x 10^6 cpm) was loaded onto a 10-25% sucrose gradient and centrifuged for 3 hr at 44,000 rpm in a Spinco SW 56 rotor at 106C.

in the presence of FdU virtually all cells contain T antigen and 70-80% of these cells are ready to start simultaneously (cellular and viral) DNA synthesis immediately upon removal of FdU inhibition by the addition of thymidine to the culture medium.

In the present experiments polyoma-infected mouse kidney cell cultures infected in the presence of FdU (15 μg/ml) were labeled with [^{3}H]-uridine (+FdU) between 26-29 hr after infection. RNA was extracted as described above and analyzed by sedimentation in sucrose density gradients. The Load profile (total cytoplasmic RNA) was similar to those shown in Figs. 1A and 2A. However, in 3 independent experiments there was slightly less radioactivity present in the 28S RNA peak as compared with the 18S ribosomal RNA peak. This may be considered as a possible late effect of the FdU inhibition, which is not yet observed at 20-23 hr after infection in the presence of FdU (5) or an effect of prolonged FdU inhibition (not observed until at least 23 hr) upon the processing and/or transport of ribosomal RNA.

In sedimentation profile of the Load (not shown), the bulk of Py RNA sediments as 2 poorly resolved peaks at 16S and 19S; a smaller fraction was consistently found sedimenting more slowly extending to the top of the gradient. In addition, there consistently appeared a small region of Py RNA sedimenting at 4-5S. This pattern of Py RNA sedimenting at 4-5S is similar to that described for RNA labeled from 20-23 hr (5).

Figure 3 shows the pattern of Oligo(dT)-retained RNA (labeled from 26-29 hr) following centrifugation in an aqueous density sucrose gradient. The distribution of total radioactivity is similar to that observed in mock infected controls, and is also very similar to that found at early times of infection (Fig. 2B). The bulk of Py RNA shows the pattern observed in the Load RNA with peaks at 16S and 19S, however, the slower sedimenting Py RNA is not present. Analysis of the Flow-through RNA reveals a pattern of total radioactivity comparable to that shown in Figs. 1C and 2C except that the 28S peak is slightly

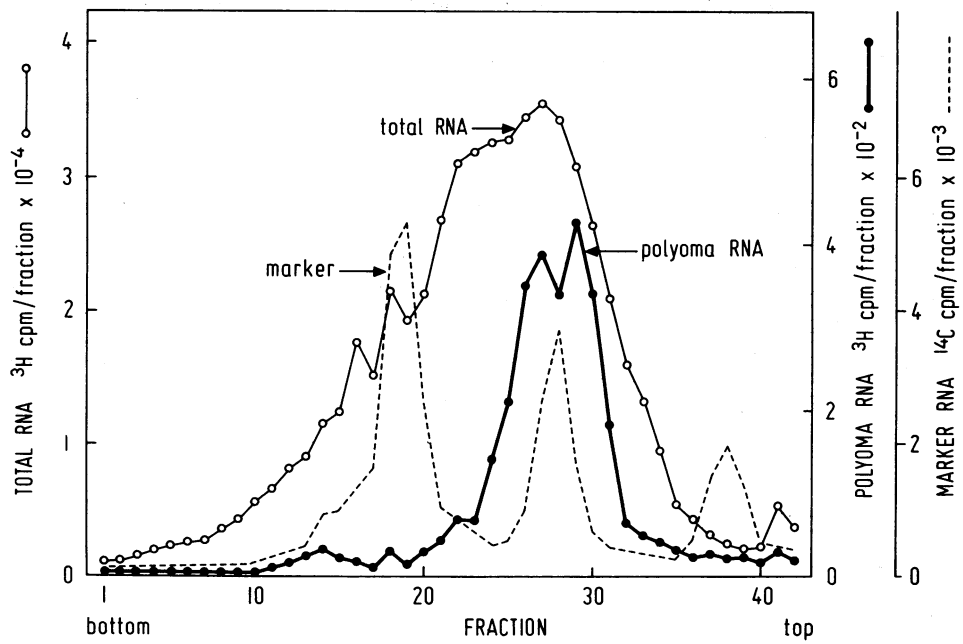

Figure 3

Sucrose gradient sedimentation analysis of Oligo(dT)-retained cytoplasmic RNA from polyoma-infected mouse kidney cells labeled with [^{3}H]-uridine (200 μCi/ml) between 26-29 hr after infection in the presence of FdU (15 μg/ml). Approximately 4.4 mg of Load RNA (10,000 cpm/μg) was applied to a 5 ml column of oligo(dT)-cellulose. Of the Oligo(dT)-retained RNA which accounted for approximately 11-12% of total, about 1 x 10^{6} cpm was mixed with 2 μg of mouse cell RNA labeled with [^{14}C]-uridine (28,000 cpm/μg) and applied to a 10-25% sucrose gradient. Centrifugation was 2.5 hr at 50,000 rpm in the Spinco SW 56 rotor at 10°C. Polyoma-specific RNA determined by hybridizations was approximately 1%.

smaller. The Py RNA, on the other hand, is mostly absent in the area where 16S and 19S would sediment, while the slow sedimenting Py RNA (4-5S) is nearly quantitatively recovered. These results suggest that only 16S and 19S Py RNA molecules contain tracts of poly(A); while the slower sedimenting Py RNA either does not contain poly(A) tracts or that the tracts of poly(A) are too short as to allow retention by oligo(dT)-cellulose.

DISCUSSION

Early in lytic infection in primary mouse kidney cell cultures prior to Py-induced cellular RNA synthesis, small amounts of polyribosome-associated Py 19S mRNA (early 19S mRNA) have been observed. "Early" 19S mRNA, with a mole-

cular weight of approximately 7 x 10^5 daltons and a coding capacity for one or more polypeptides with a total molecular weight of 70,000, is thought to be the contiguous transcript of the early region of Py DNA (5,25). The experimental observations presently available lead to the hypothesis that early 19S mRNA may contain most of the information required for induction of lytic and abortive infection and for initiation and maintenance of the virus-specific transformed phenotype. To reconcile the small amount of information with the large spectrum of biological effects exerted by Py (and SV40), the hypothesis has been put forward that the early 19S mRNA may code for a polyfunctional protein (pleiotropic effector) (5) which induces phenotypic modulation of the host cell. However, in order to test this hypothesis it will be necessary to isolate 19S mRNA, to translate *in vitro*, and eventually to test the polypeptide(s) specified for their chemical, immunological, and biological properties. The results reported in this paper show that early 19S mRNA contains poly(A) tracts which allow its quantitative recovery in an intact form. In preliminary studies purified Py mRNA was obtained following hybridization and elution to Py DNA fixed to membrane filters using a modification of the procedure described by Weinberg *et al*. (7).

Late in lytic infection, when viral DNA synthesis is at a maximum, two classes of polyribosome-associated Py RNAs are found; a major component (80-90%) and a minor component (10-20%) with sedimentation coefficients of 16S and 19S (late 19S RNA) respectively as determined in aqueous sucrose density gradients. Since both classes (16S and 19S) are present in cytoplasmic polyribosomes in an association which is sensitive to treatment with EDTA, they are assumed to be presumptive mRNA molecules (4). The class of 16S mRNAs is thought to specify polyoma capsid proteins while the biological significance of late 19S RNA remains unknown.

In addition, during infection under conditions where DNA synthesis is inhibited with FdU, two classes of cytoplasmic polyribosomal Py RNA sedimenting

at 16 and 19S were observed after the onset of cellular RNA synthesis (5). Several possibilities for the transcription of (late) 16S RNA are presently being investigated: (1) the FdU block is not entirely effective and a small amount of DNA replication takes place; however, in a series of experiments in which cultures were infected and labeled with [^{3}H] cytidine in the presence of FdU, no detectable polyoma DNA component I was observed in EtBr-CsCl density gradients. (2) the 16S RNA is a breakdown product of 19S RNA and represents only early sequences; and (3) DNA replication is not a prerequisite for late transcription. The nature of these classes is not understood and is presently being investigated. However, in experiments performed with BSC-1 cultures infected with SV40 in the presence of FdU in which no significant DNA replication was observed (Rosenthal, Brown, and Khoury, manuscript submitted for publication) the 16S RNA was identified to be late 16S RNA which hybridized to the plus strand of SV40 DNA. Similar observations have recently been made by others in polyoma virus infected cells grown in the presence of FdU, (Beard et al. and Turler and Weil, personal communications).

The results reported in this paper as determined by oligo(dT)-cellulose column chromatography, show that the classes of Py RNA synthesized during lytic infection either in the presence or absence of FdU contain tracts of poly(A). From analysis in aqueous sucrose density gradients, and also in denaturing Me_2SO gradients, the viral species isolated by oligo(dT)-cellulose column chromatography appear to be intact (Rosenthal, unpublished results). According to hybridizations with Py DNA, late in lytic infection, about 10% of the total oligo(dT)-retained radioactive RNA is Py RNA, while early in lytic infection Py RNA accounts for only 0.02-0.03% of the total. Late in lytic infection in the presence of FdU, Py RNA accounts for approximately 1% of the total radioactive mRNA retained by oligo(dT)-cellulose.

The sedimentation profiles of total Oligo(dT)-retained mRNAs isolated early in lytic infection or late during lytic infection in the presence of FdU were

indistinguishable from those of mRNAs extracted from mock infected parallel cultures. Since lytic infection even in the presence of FdU leads to the activation of a considerable number of host cell functions, it is to be expected that more refined methods of analysis would reveal differences in the mRNA patterns. In contrast Oligo(dT)-retained RNA extracted late during lytic infection shows an altered sedimentation pattern which is due either to the presence of considerable amounts of Py RNA (10% of total) or to a decreased production of cellular mRNA as a result of intranuclear synthesis and processing of relatively large quantities of Py RNA.

The results described in this report together with the observation that cytoplasmic Py RNA in Py-transformed hamster cells (BHK) contain poly(A) tracts (Maxwell, unpublished results) show that all presently known classes of Py mRNAs can be isolated by oligo(dT)-cellulose chromatography. Preliminary studies have shown that Oligo(dT)-retained RNAs are excellent templates for in vitro synthesis of proteins (Rosenthal and Spahr, unpublished results). In this context it should be mentioned that by using SV40 RNA retained on oligo(dT)-cellulose (26) and poly(A) sepharose (27) the cell-free translation of SV40 mRNA into the major capsid polypeptide VP-1 has been reported.

In the subsequent report (Rosenthal et al., this volume) it will be shown that newly synthesized intranuclear "giant" Py RNA molecules synthesized late in the lytic infection (+FdU) contain poly(A) tracts. This should allow one to study the structural properties and the fate of poly(A)-containing intranuclear "giant" RNA molecules and to determine whether they are precursors of the polyribosomal mRNAs.

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*Present address: Department of Microbiology, Georgetown University, Schools of Medicine & Dentistry, Washington, DC 20007, USA.

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