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New Nuclear Ribonucleic Acid in Cell Cultures Infected with Polyoma Virus

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The technical basis for the study of the site of maturation of polyoma virus (PYV) in mouse embryo (ME) cells by direct cell fractionation was established in a previous study (6). These techniques provided a rigorous separation of nuclei and cytoplasm, and the results agreed with earlier reports (13) that the hemagglutinin and complete PYV were formed in the nucleus of the host cell. Evidence for the presence of ribonucleic acid (RNA) specific for PYV has been shown in infected mammalian cell cultures (2, 7). In the present study, using these cell fractionation techniques, we found that a PYV-specific RNA appears in the nuclei from about 12 hr after infection.

ME cultures were infected with PYV at a multiplicity of 50 plaque-forming units per cell (6). At 0, 4, 8, 12, and 16 hr after infection, the culture medium on a set of 30 dishes was replaced with one containing 10 μc of uridine-5-^3H per ml and was incubated at 37 C for 30 min. The radioactive medium was removed, the cells were washed, harvested, and separated into the nuclear and cytoplasmic fractions with cold 0.1% (v/v) Tween 80 solution (5), and the nuclear RNA and the cytoplasmic RNA were extracted and purified (8). The purified RNA samples were overlaid on a 5 to 20% sucrose (w/v; in 0.25 M NaCl, 0.005 M $MgCl_2$, 0.05 M acetate buffer, *p*H 5.2) density gradient and were centrifuged at 100,000 $\times$ *g* for 3 hr in a Spinco SW-39 rotor. The fractions were collected, and the absorbance at 260 nm and the radioactivity were measured.

Figure 1A shows the results of sedimentation of cytoplasmic RNA from uninfected cells. Identical patterns were obtained with cytoplasmic RNA of infected cells through the first 12 hr after infection, and only 4*S* RNA was found to be appreciably labeled.

The nuclear RNA from both infected and uninfected cells (Fig. 1B, C, and D), however, showed significant labeling of many components (55*S*, 45*S*, 35*S*), in agreement with published data for other cultured cells (1, 11). By 12 hr after infection (Fig. 1D), several differences were found in the sedimentation pattern of the radioactive RNA, when compared to the uninfected nuclear RNA (Fig. 1B) and the nuclear RNA 4 hr after infection (Fig. 1C). In particular, new 10*S* and 20*S* RNA components appeared and the 16*S* component was significantly reduced.

To determine whether the radioactive nuclear RNA components were related to cellular deoxyribonucleic acid (DNA) or viral DNA, complementarity tests were carried out. PYV stock was prepared as described by Winocour (14) and uninfected ME cell nuclei were prepared as described previously (6). DNA was extracted from the stocks by Marmur's method (9). For some experiments, the DNA from the PYV stock was further purified by sedimentation in a sucrose gradient to isolate the 20*S* component (3).

The DNA was denatured by heating at 100 C for 10 min in SSC (0.15 M NaCl, 0.015 M sodium citrate, *p*H 7.0) which had been diluted 50-fold in distilled water. Each radioactive RNA fraction (less than 2 μg) to be tested from a peak shown in Fig. 1 was divided into three equal samples. (i) One sample was used to determine the total trichloroacetic acid-insoluble RNA present. (ii) To a second sample, 10 μg of denatured PYV or ME cell DNA was added, and the total ribonuclease-resistant, trichloroacetic acid-insoluble RNA after complexing was determined. (iii) To compensate for normal ribonuclease-resistant RNA, a third sample was treated with ribonuclease without DNA.

All of the above samples were diluted to 1.0 ml with 2$\times$ SSC and were heated to 67 C for 18 hr followed by slow cooling to room temperature over a period of 24 hr. A stock ribonuclease solution was heated at 80 C for 10 min to inactivate deoxyribonuclease. This ribonuclease solution was added to the second and third samples to a concentration of 5 μg/ml after annealing the samples and was incubated at 25 C for 60 min (15). Then the samples were precipitated with cold trichloroacetic acid and

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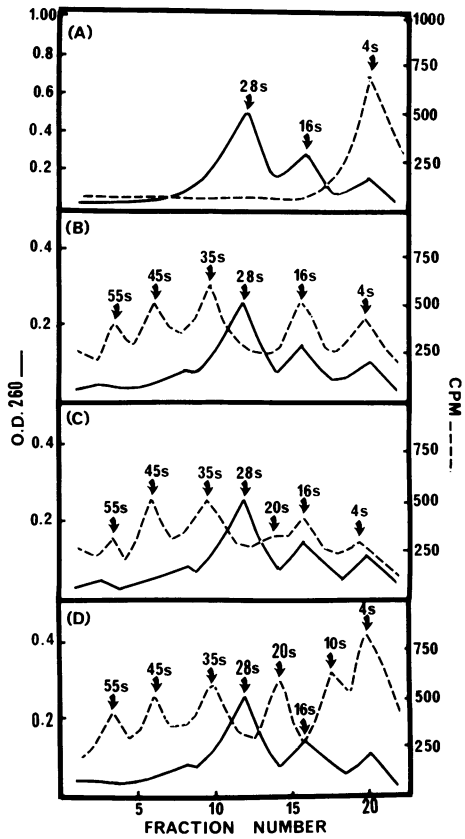

FIG. 1. *Sucrose density gradient sedimentation analysis of nuclear and cytoplasmic RNA of infected and uninfected cells. At 4-hr intervals after infection, the cells were labeled by 30 min of incubation at 37 C with 10 μc of uridine-5-³H per ml in the growth medium, and the nuclear and cytoplasmic RNA samples were prepared. The RNA samples were overlaid on a 5 to 20% sucrose density gradient and were centrifuged at 100,000 × g for 3 hr in a Spinco SW-39 rotor; the fractions were collected by puncturing the bottom of the centrifuge tube. Each part of the figure represents the average of the data from three independent experiments. The solid line presents the data for the absorbance of the fractions at 260 nm, and the broken line represents the radioactivity measured by scintillation counting of membrane filters (Millipore Corp.) on which the insoluble precipitates were collected by suction filtration after the addition of an equal volume of cold 10% trichloroacetic acid to the fractions. (A) Data from the cytoplasmic RNA of uninfected cells; (B) nuclear RNA of uninfected cells; (C) nuclear RNA at 4 hr after infection; and (D) nuclear RNA at 12 hr after infection. The S values were calculated using HeLa cell ribosomal RNA (28S and 16S) as markers (10).*

collected on membrane (Millipore Corp., Bedford, Mass.) filters (12) for radioactive measurements.

Table 1 shows the results of the complementarity experiments. The new 20*S* nuclear RNA component annealed to at least 17% with the PYV DNA but only to 4% with cell DNA. The RNA of other components (55*S*, 45*S*, 35*S*, and 10*S*) annealed to PYV DNA only to 7% or less, whereas they annealed to cell DNA to at least 20%, with the exception of the 35*S* component at 12 hr after infection.

These results suggest that, in addition to the appearance of the new virus-specific RNA in the nucleus after infection, there are also detectable changes in the types of nuclear RNA which normally mature into ribosomes. Two notable changes are the disappearance of the 16*S* component and the decreased capacity of the 35*S* component to anneal to cell DNA. The disappearance of the 16*S* RNA from the nucleus of mammalian cells in the presence of cycloheximide,

TABLE 1. *Complementarity tests of nuclear RNA fractions with purified PYV DNA and with purified cell DNA*

Sedimentation value of fractions[b]	Per cent complementation[a]		
	Cell DNA[c]	PYV DNA[c]	20*S* PYV DNA[d]
Uninfected cells			
55*S*	20		
45*S*	28	7	
35*S*	35	5	
16*S*	17		
Infected cells (12 hr)			
55*S*	19	3	
45*S*	21	7	
35*S*	7	3	4
20*S*	4	30	17
10*S*	21		0

[a] Annealing was carried out as described in the text and the radioactive counts of the RNA were used in the following calculation. Per cent complementation between RNA and DNA = (counts/min in "hybrid") − (counts/min in "native")/(counts/min in total) × 100, where (i) "hybrid" is the ribonuclease-resistant radioactivity in RNA after annealing as described in the text for the second sample; (ii) "native" is the ribonuclease-resistant radioactivity in RNA without annealing, as described in the text for the third sample; (iii) total is the total trichlroacetic acid-insoluble radioactivity in RNA as described in the text for the first sample.

[b] Sedimentation values of radioactive fractions shown in Fig. 1B and 1D.

[c] ME cell DNA and PYV DNA purified by Marmur's method as described in the text.

[d] The 20*S* PYV DNA was isolated by velocity sedimentation in a sucrose gradient.

an antibiotic used to stop protein synthesis, was previously noted by Ennis (4). Since the annealing was measured with pulse-labeled RNA fractions, the decreased ability of the 35*S* RNA to hybridize could be explained by a decreased specific activity if the 35*S* RNA was not maturing into ribosomal RNA.

Pulse-chase experiments are currently in progress to determine if this new virus-specific RNA can be isolated from polysomes collected from the cytoplasmic fraction and hence can play a role in the synthesis of virus proteins.

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