NUCLEUS-ASSOCIATED RNA IN MEASLES VIRUS-INFECTED CELLS

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Summary. Measles virus RNA was found in the nuclear fraction of infected Vero cells. 24-hr labeling periods revealed heterogeneously sedimenting 15-50 S RNA associated with a membrane-containing particulate. Viral RNA isolated after shorter labeling periods was larger in size (30-50 S) and associated with both nucleoplasmic and particulate fractions.

Development of measles virus in cultured human cells was examined by Rapp et al (1) using the immunofluorescent technique. They found specific fluorescence which tended to localize first in the perinuclear region and later spread throughout the cytoplasm. Discrete aggregates of antigen were also seen in the nuclei late in infection. Nakai et al (2), using electron microscopic techniques, found that nucleoprotein tubules accumulated in the cytoplasm of infected BSC-1 cells and also inside the nuclei of some cells late in the infective cycle.

To determine whether the presence of nucleoprotein antigen and tubules in the nucleus reflects an accumulation site for measles RNA as well as virus protein, we examined nuclear and cytoplasmic RNA extracts of Vero cells infected with the Rapp virulent strain of measles virus (3). Fig. 1 shows sedimentation profiles of RNA extracts from cells exposed to ^{3}H-uridine from 12 to 36 hr post infection. The radioactivity associated with acid-precipitable RNA in nuclear extracts from infected cells was from 1.5 to 3 times that of uninfected controls in repeated experiments.

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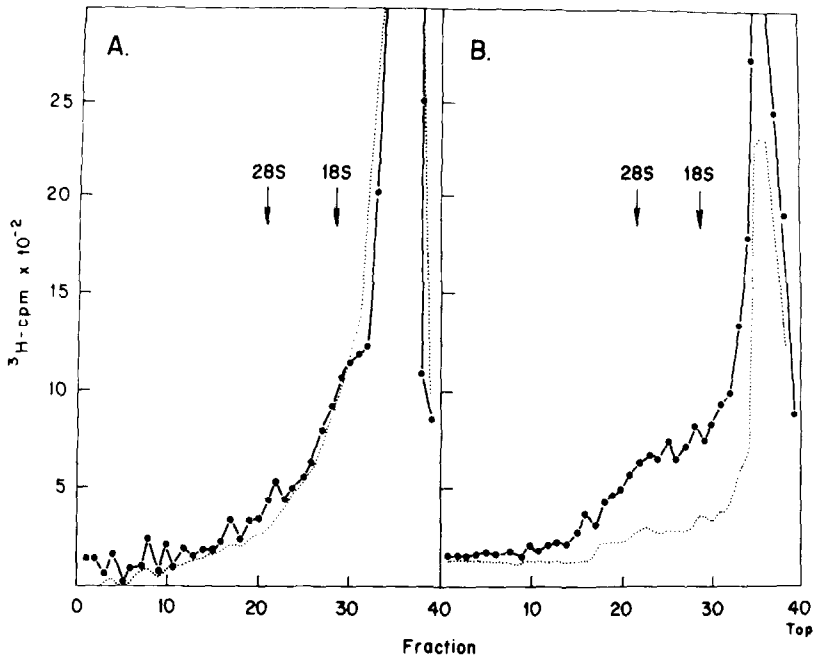

Figure 1. Sedimentation analysis of RNA extracted from cytoplasm and nuclei of measles virus infected (———) and uninfected (.....) Vero cells.

A. Cytoplasm: 3 x 10^6 control cells or cells 23 hr post infection were exposed to 5 μg/ml of actinomycin D for 60 min prior to addition of 50 μCi/ml of ^{3}H-5-uridine. After 24 hr in the presence of drug and label, cells were lysed in 0.5% Nonidet-P 40 in NET buffer (.01 M Tris, pH 8.5, 0.1 M NaCl, 0.001 M EDTA) and nuclei were separated from cytoplasm by centrifugation (2000 rpm, 2 min). Cytoplasmic RNA was extracted with 2% sodium dodecyl sulfate (SDS) in the presence of 20 μg/ml polyvinyl sulfate. The extract was layered directly onto 15-30% sucrose density gradients and centrifuged 120 min, 50,000 rpm, at 15° C in an SW 50L rotor. Fractions were collected on filter paper discs, acid precipitated and counted as previously described (4).

B. Nuclei washed in NET buffer were suspended in high-salt buffer (0.5 M NaCl, 0.05 M MgCl, 0.01 M tris, pH 7.4) and released DNA was digested with 40 μg DNase. After 20 min at 37° C, total nuclear RNA was precipitated from the mixture with 2.5 volumes of ethanol. RNA was resuspended in NET buffer containing 2% SDS and 20 μg/ml polyvinyl sulfate. Samples were layered onto gradients and centrifuged as described for cytoplasmic RNA.

It sedimented heterogeneously with coefficients estimated at from 15 S to 50 S. In contrast, there was little radioactivity in infected cytoplasm above control levels and the 20 S, 27 S, 35 S and 52 S RNA size classes observed in this system in cytoplasmic extracts of pulse-labeled cells (4) could not be resolved.

Nuclei used in the experiment represented in Fig. 1 were not detergent-cleaned (5) prior to extraction. If nuclei were first treated with tween 80-desoxycholate to strip off the outer nuclear membrane (6), all the viral radioactivity associated with the nuclei was lost. This suggested that the perinuclear membrane might be a site of measles virus RNA accumulation. However, we found that detergent treatment of infected and control cell nuclei resulted in the loss of radioactively labeled thymidine as well, and we could not exclude the possibility that loss of viral RNA was due to leakage from stripped nuclei.

To differentiate between RNA associated with nucleoplasm and nuclear membrane-containing particulate fractions, nuclei from cells exposed to ^{3}H-uridine for 24 hr in the presence of actinomycin D were separated from cytoplasm and disrupted as described in the legend for Fig. 1. In this experiment the DNase treated nuclear extract was divided into a low-speed pellet (2,000 rpm, 10 min), a high-speed pellet (13,500 rpm, 15 min) and a supernatant fraction (nucleoplasm). The pellets were solubilized in buffer containing 2% sodium dodecyl sulfate (SDS) and samples from replicate cultures were counted. The nucleoplasm was adjusted to contain 2% SDS and sampled directly onto filter discs. As shown in Table 1, all of the radioactivity attributable to viral RNA was associated with the particulate fractions. This was in marked contrast to control nuclear radioactivity which was predominantly in the nucleoplasm and showed a distribution comparable to incorporated ^{3}H-thymidine under the same conditions. The data appear to eliminate the nucleoplasm as an accumulation site for viral RNA and are compatible with a viral perinuclear membrane site. However, because the particulate fractions both contain nucleolar as well as membrane components[1], the precise viral site remains

[1]Nuclei from control cell preparations labeled for 30 min with ^{3}H-uridine were fractionated as described. The particulate fractions were separated on sucrose-D_2O gradients covering a density range of 1.15 to 1.30 gm/ml. Both low and high-speed pellets contained low-density membrane fractions associated with 12-23% of the rapidly-labeled nucleolar RNA and a heavy fraction ($>$1.30 g/ml) containing most of the rest.

Table 1. Distribution of ^{3}H-uridine labeled RNA in nuclear fractions from actinomycin-treated infected and uninfected Vero cells.

	Uninfected		Infected		Viral[1]	
	^{3}H-cpm	Percent	^{3}H-cpm	Percent	^{3}H-cpm	Percent
Low-speed pellet (2000 rpm)	1,715	9	7,834	32	6,119	77
High-speed pellet (13,500 rpm)	3,026	14	4,889	20	1,863	23
Nucleoplasm (supernatant)	17,396	77	11,814	48	--	--

[1]Infected - Uninfected

unknown. The exact amount of virus-specific nuclear RNA is also uncertain, although it is clear that a significant amount becomes associated with the nucleus over a 24-hr labeling period. In the experiment shown in Table 1, there were about 8000 cpm above control levels in nuclear particulate fractions from infected cells which would represent 63% of total viral RNA in the cells. However, when total nuclear counts were compared, there were only 2,500 cpm above control levels representing 33% of viral RNA. Specific hybridization experiments are needed to clarify the actual distribution of viral RNA.

To see whether pulse-labeled measles RNA is also associated with the nuclear particulate fraction, cells infected with diluted passage virus (4) were exposed to ^{3}H-uridine for 90 min in the presence of actinomycin D and nuclei were fractionated as described above. 10% of viral RNA was recovered in the nucleus--5% in the two particulate fractions and 5% in the nucleoplasm. Although the amounts found were small, the size distributions of viral RNA associated with the nuclear particulates and supernatant fraction were indistinguishable from each other but distinct from that of viral cytoplasmic RNA (Fig. 2). In cytoplasmic extracts, 20 S

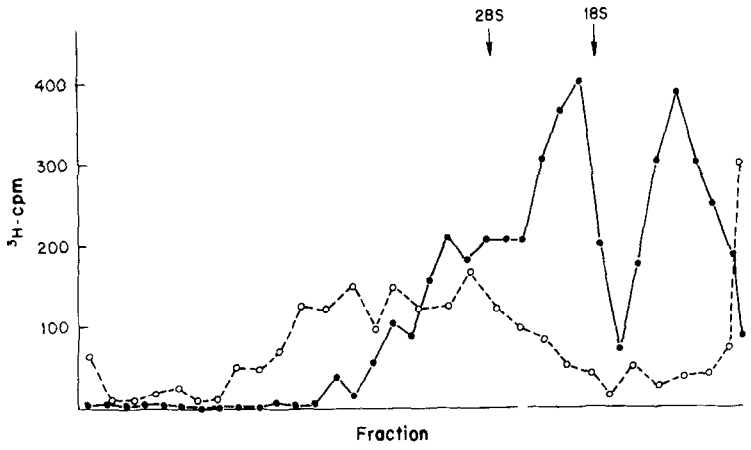

Figure 2. Sedimentation analysis of viral RNA in cytoplasm (.——.) and in the low-speed particulate fraction from nuclei (o---o) of infected Vero cells[1].

3 x 10^7 control cells or cells 48 hr post infection were exposed to 50 µg/ml of actinomycin D for 30 min prior to addition of 100 µCi/ml of ^{3}H-5-uridine. After a 90-min labeling period, cells were washed with ice-cold medium and nuclei were separated from cytoplasm as described in the legend to Fig. 1. Particulate and nucleoplasmic fractions were obtained as described in the text. Gradients were centrifuged for 90 min in an SW 50.1 rotor at 15° C.

[1]For clarity of presentation, only the counts above control levels have been plotted. Radioactivity in nuclear extracts from uninfected cells showed a peak only at the top of the gradients.

RNA was the predominant component and 50 S was seldom observed. In contrast the larger RNAs were regular and predominant components of the nuclear extracts.

The large amount of viral RNA associated with nuclei after long labeling periods suggests nuclear accumulation. The association of this RNA with particulate structures and its release by detergent suggest that the site of accumulation may in fact be perinuclear. Following a short labeling period, viral RNA with a size distribution compatible with replicating forms (7) was found in both the nucleoplasm and the particulate. The nuclear aggregates of measles antigen and the sensitivity of paramyxovirus replication to cordycepin (8) have raised the possibility of a

nuclear phase in the synthesis of these viruses. Our results are compatible with that possibility.

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