THE DISTRIBUTION OF VIRUS-SPECIFIC RNA BETWEEN INFORMOSOME COMPONENT AND POLYRIBOSOMES IN THE EXTRACT OF NDV-INFECTED CELLS

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1. Introduction

In the previous communication [1] we have described an informosome-like 40–45 S virus-specific component in cytoplasmic extract of Newcastle Disease Virus-infected cells. In this report data on the distribution and possible redistribution of virus-specific RNA between the informosome component and polyribosomes are presented.

2. Materials and methods

The methods for the cultivation and labeling of the cells as well as for the preparation and analysis of cytoplasmic extracts were the same as in the previous communication [1].

3. Results and discussion

The major part of virus-specific preribosomal material in the extracts of NDV-infected cells seems to be represented by viral polyribosomes. This material is sensitive to EDTA and after deoxycholate treatment its buoyant density is characteristic for ribosomal structures (fig. 1). Viral nucleocapsid does not contaminate the polyribosomes; the major part of it may be found in the 15,000 *g* pellet after deoxycholate treatment of the latter. Virus-specific RNA in the postribosomal zone is located in the informosome component.

The distribution of virus-specific RNA between postribosomal material and polyribosomes changes in the course of infection. The relative amount of the RNA in the postribosomal zone sharply increases during the 10th hr of the infectious cycle (fig. 2).

Further data on the redistribution of viral RNA were obtained with the use of the inhibitors of protein synthesis. In NDV-infected cells viral-RNA synthetase is stable [2] and the synthesis of viral RNA may be studied in the absence of protein synthesis. When the virus-specific RNA was labeled in

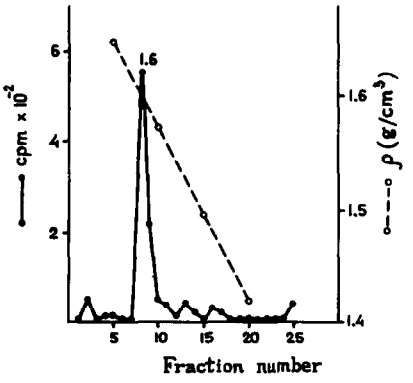

Fig. 1. The buoyant density of virus-specific polyribosomal material after deoxycholate treatment of cytoplasmic extract. The cytoplasmic extract of NDV-infected cells was treated with sodium deoxycholate in final concentration 0.5%, fixed with formaldehyde and fractionated in 15–30% sucrose gradient. Preribosomal (> 100 S) material was analysed in a CsCl density gradient (3 X 5 rotor of an M.S.E. Superspeed-50 ultracentrifuge, 36,000 rpm, 16 hr).

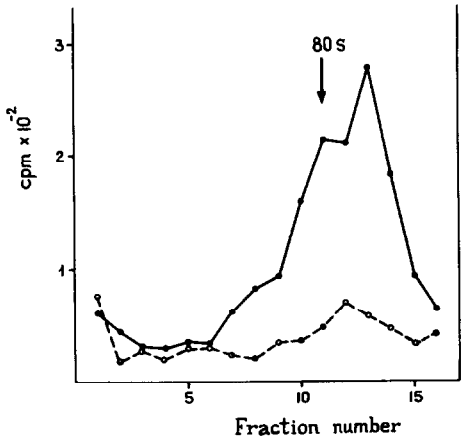

Fig. 2. Sedimentational distribution of virus-specific material in the extracts of NDV-infected cells at different times after infection. The cells were disrupted 9 or 10 hr post infection (p.i.). Cytoplasmic extracts were layered on top of 15–30% sucrose gradients and centrifuged for 2 hr at 25,000 rpm in 3 X 23 rotor. ○ - - - ○, 9 hr p.i.; ●——●, 10 hr p.i.

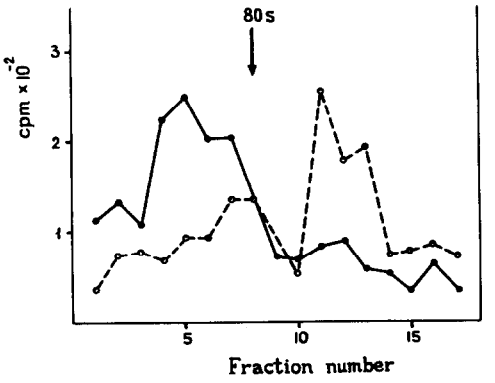

Fig. 3. The distribution of virus-specific RNA in the structures of cytoplasmic extract after exposure of the cells to cycloheximide. Cycloheximide was added to culture fluid 1 hr before the addition of ^{3}H-uridine. The cytoplasmic extract was analysed in 15–30% sucrose gradient (20,000 rpm, 10 hr). ●——●, cycloheximide-treated cells; ○ - - - ○, cells not treated with cycloheximide.

the presence of cycloheximide (50 μg/ml), most of the labeled material was found in the polyribosomal zone (fig. 3). This change in the pattern of distribution was never observed if puromycin was used instead of cycloheximide. For this reason the redistribution seems not to be a direct result of the inhibition of protein synthesis. An increase in the amount of polyribosomes in cycloheximide-treated cells has been reported [3]. The structures containing NDV-specific RNA in cycloheximide-treated cells seem to be true polyribosomes, as they are sensitive to EDTA treatment.

Virus-specific RNA may be transferred into cycloheximide-induced polyribosomes directly from its site of synthesis, or it may pass there from the informosome component. The latter situation is possible in view of the data obtained under 'chase' conditions, when the increase in the amount of labeled RNA was negligible but the redistribution under the action of cycloheximide was quite obvious [4].

The overall data (see also [4]) seem to indicate an interrelationship between the RNA of informosome component and that of viral polyribosomes and therefore suggest a functional significance of the RNA of virus-specific informosomes.

References

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