VIRUS-SPECIFIC INFORMOSOME COMPONENTS IN THE EXTRACTS OF NEWCASTLE DISEASE VIRUS INFECTED CELLS

V.G. ZASLAVSKY, V.M. ZAIDES, M.Ya. VOLKOVA, N.V. KAVERIN and A.G. BUCRINSKAYA
Ivanovsky Institute of Virology, Academy of Medical Sciences, Moscow, USSR

Received 26 December 1970
Revised version received 3 February 1971
Original figures received 19 February 1971

1. Introduction

Virus-specific RNA in paramyxovirus-infected cells consists predominantly of complementary ('minus') strands [1, 2] with sedimentation coefficients of 18 S, 22 S and 35 S [2, 3]. The large proportion of free 'minus' strands and their recovery from polyribosomal material suggests that they have a messenger function in the synthesis of viral proteins [1, 2]. Virus-specific RNA in cell extracts is present not only in the polyribosomal zone but also in the 'light' material at the top of sucrose gradient [2, 4]. The data presented in this paper suggest that this postribosomal material as well as a part of pre-ribosomal components have properties of the informosomes [5, 6].

2. Materials and methods

Chick embryo cell monolayer cultures were infected with Newcastle Disease Virus (NDV), Beaudette strain. For the detailed description of the infection and labeling technique and for analytic procedures see [7]. Cells were treated with actinomycin D (2 μg/ml), labeled with ^{3}H-uridine (3 μCi/ml, 0.3 Ci/mmole or 50 μCi/ml, 20 Ci/mmole), suspended in a hypotonic buffer (0.01 M triethanolamine-HCl pH 7.8; 0.01 M KCl; 0.003 M Mg^{2+}) and disrupted in a Dounce homogenizer. Cytoplasmic extracts were fractionated in sucrose density gradients. Fractions of the gradient were fixed with formaldehyde and analysed in preformed CsCl density gradients [8, 9]. For RNA–RNA hybridization unlabeled viral RNA was obtained from purified [10] NDV and the ar nealing procedure was performed as described by Kingsbury [1].

3. Results and discussion

In the cytoplasmic extracts the virus-specific l is distributed in the polyribosomal zone of the su density gradients and in a narrow postribosomal The postribosomal material has a sedimentation

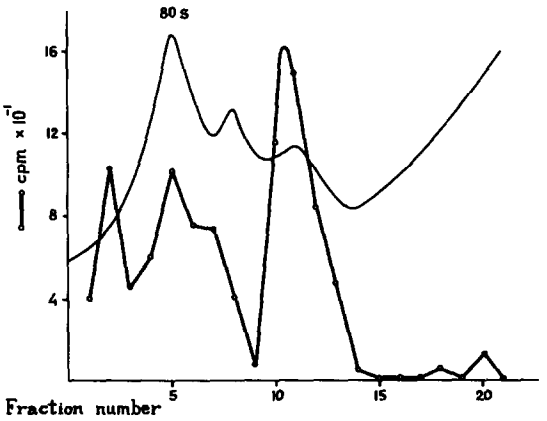

Fig. 1. Fractionation of cytoplasmic extract of NDV-inf cells in sucrose density gradient. The formaldehyde-fixe cytoplasmic extract was layered on top of 15–30% sucr gradient containing 4% formaldehyde and centrifuged f 11.5 hr at 22,000 rpm (3 × 23 bucket rotor of M.S.E. S speed-50 ultracentrifuge). Fractions were collected thro a flow cell in a recording spectrophotometer (λ = 254 n Aliquots were taken for counting in a Packard TriCarb Spectrometer.

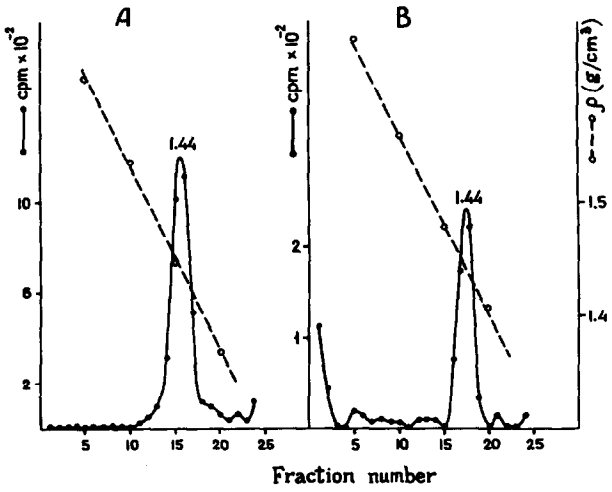

Fig. 2. Buoyant density of virus-specific material from 40 S peak. (a) with formaldehyde fixation of cytoplasmic extract. Fraction nos. 10 and 11 from the sucrose gradient (fig. 1) were pooled, dialysed and analysed in a preformed CsCl gradient (3 X 5 rotor of M.S.E. Superspeed-50, 16 hr at 35,000 rpm). (b) with formaldehyde fixation after fractionation in 5–20% sucrose gradient (4.5 hr at 25,000 rpm). Fractions were fixed with 1/10 volume of 40% formaldehyde. Material from 40 S peak was analysed in a CsCl gradient.

of about 40–45 S (fig. 1) and a buoyant density 1.42–1.44 g/ml (fig. 2).

This value was obtained both when cytoplasmic extract had been fixed with formaldehyde prior to fractionation in sucrose gradient (fig. 2a) and when fixation of the fractions from sucrose gradient was performed (fig. 2b). The same buoyant density value was obtained (fig. 3) when the cells were broken in a buffer containing 0.15 M KCl, i.e. at ionic strength which is thought to be characteristic for the intracellular environment[11].

Sodium dodecylsulfate (SDS) treatment of the postribosomal material releases virus-specific 18 S RNA (fig. 4). This RNA is complementary to RNA of the mature virus (table 1).

Ribonucleoprotein structures with ρ = 1.40–1.45 g/ml containing non-ribosomal (presumably messenger) RNA have been described and termed 'informosomes' [5, 6]. Sedimentation rate of informosomes is usually 2–2.5 times higher than that of the corresponding RNA [12]. The 40 S virus-specific structure has the principal characteristics of the informosome.

There is some evidence that other virus-specific informosome components are also present in the

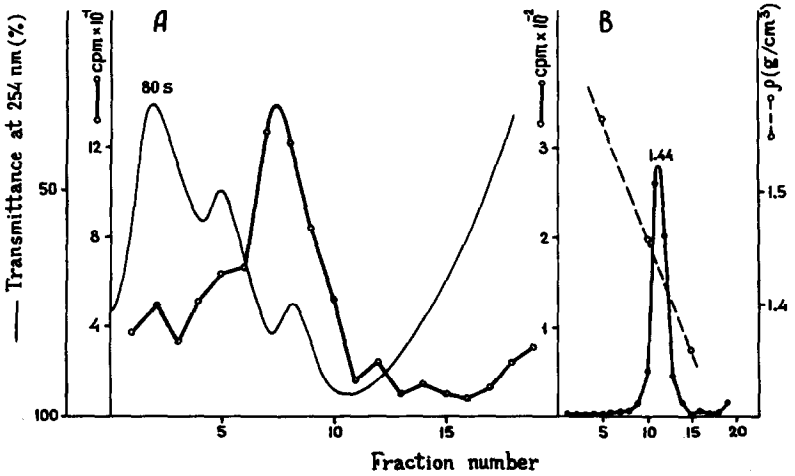

Fig. 3. Sedimentation and density distribution of informosome component from 0.15 M KCl-cell extract. Cells were disrupted in standard buffer with elevated (0.15 M) concentration of KCl. Cytoplasmic extract was layered on top of 15–30% sucrose gradient containing 4% of formaldehyde and centrifuged for 11 hr at 25,000 rpm in 3 X 23 rotor. (a) sedimentation pattern in sucrose. (b) analysis of material of fractions NN 7 and 8 from *a* in CsCl gradient.

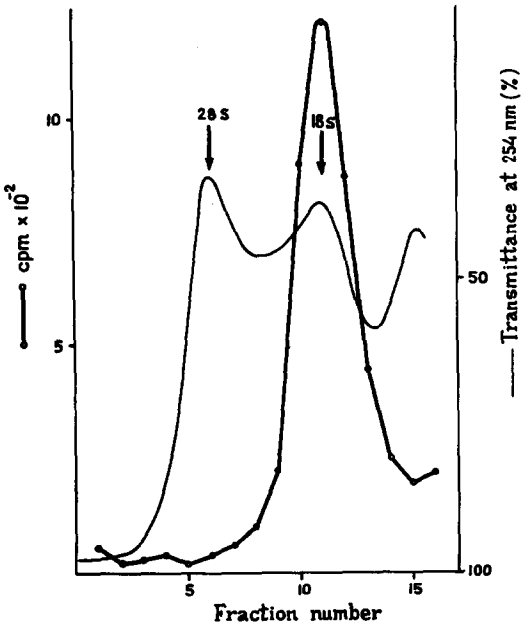

Fig. 4. Sedimentation of virus-specific RNA obtained from 40 S component. Cytoplasmic extract was fractionated in 15–30% sucrose gradient. Postribosomal material was sedimented at 120,000 *g* for 1.5 hr, resuspended in STE (0.1 M NaCl; 0.01 M tris-HCl pH 7.0; 0.001 M EDTA), SDS was added to 1%. The material was layered on top of 5–20% sucrose gradient and centrifuged in 3 X 23 rotor for 10.5 hr at 23,000 rpm.

Table 1
Hybridization of RNA extracts from postribosomal component with RNA of NDV.

Unlabeled NDV RNA (μg)	^{3}H-count per sample (cpm)		Percentage of ^{3}H-RNA annealed
	before RNase digestion	after RNase digestion	
8	720	587	81.5
4	740	502	67.8
0	428	16	3.7

Cytoplasmic extract from NDV infected cells was fractionated in 15–30% sucrose density gradient (3 X 23 rotor, 25,000 rpm, 4°, 2 hr). Postribosomal material was sedimented and resuspended in STE. RNA was extracted with SDS-phenol mixture and used for hybridization with RNA of purified NDV.

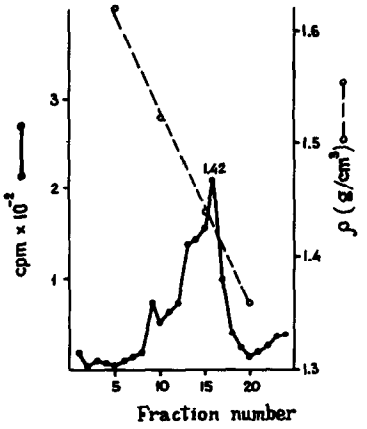

Fig. 5. Buoyant density distribution of virus-specific 90–100 S material. For conditions of fractionation in sucrose density gradient see fig. 2b. Fractions of 90–100 S zone were pooled, dialysed and centrifuged in CsCl gradient.

cytoplasmic extract. When preribosomal (90–100 S) material was analysed in CsCl density gradient, a large part of it banded at $\rho = 1.42$ (fig. 5). A fractionation of formaldehyde-fixed cytoplasmic extract in CsCl followed by a sedimentation analysis of the peak fraction with $\rho = 1.43$ revealed two components with sedimentation rates 40 S (the main component) and 90 S (the additional component) (fig. 6). It had been shown that beside 18 S RNA in NDV-infected cells other classes of viral complementary RNA (22–35 S) are present [2]. If they are able to form informosomes the latter should have, at least in part, the sedimentation rate of about 90–100 S.

The presented data demonstrate the presence in the cytoplasmic extracts of NDV-infected cells of informosome-like components of two kinds. The predominant class (40 S) contains 18 S virus-specific RNA complementary to RNA of mature virus. The 'heavy' component (90 S) presumably contains larger virus-specific RNA.

Virus-specific informosomes were first described for a DNA-virus [9]. Recently similar structures were described for poliovirus [13]. The peculiarity of the components described in this paper lies in the fact that it contains a 'minus' strand of viral RNA. Some data on their possible functional significance (at least for their ribonucleic acid moiety) are presented in [14].

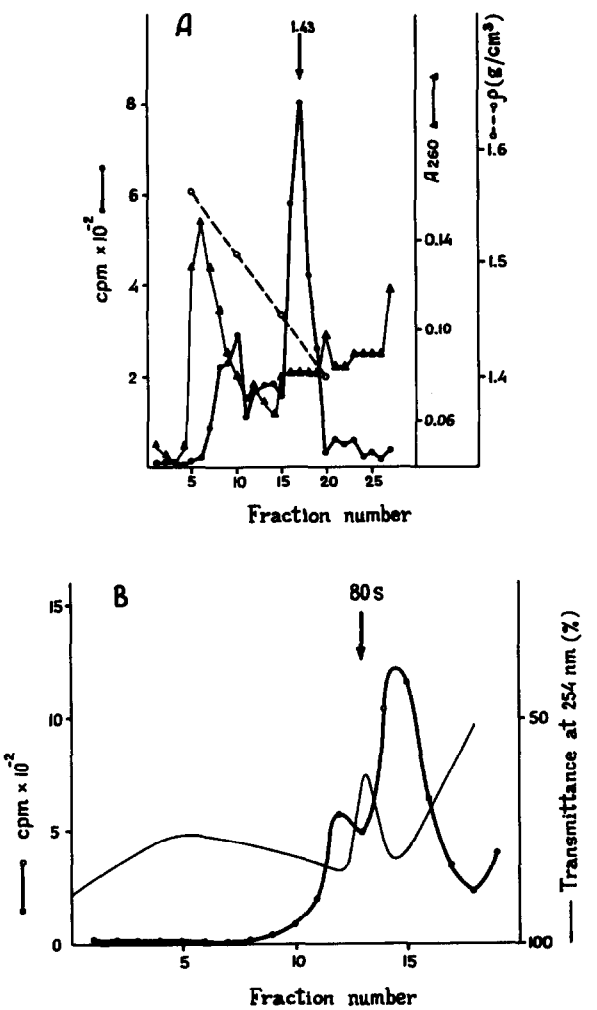

Fig. 6. Fractionation of cytoplasmic extract in CsCl density gradient and sedimentation analysis of the material of ρ = 1.43 g/ml. Cytoplasmic extract was fixed with 8% formaldehyde and fractionated in preformed CsCl density gradient (18 hr at 36,000 rpm, 3 X 5 rotor. ^{3}H-Uridine incorporation was measured in 0.25 ml aliquots (a). Fraction no. 17 was dialysed, layered on top of 15–30% sucrose gradient and centrifuged for 3 hr in 3 X 23 rotor at 25,000 rpm. Chick embryo ribosomal material was used as a marker (b).

References

[1] D.W. Kingsbury, J. Mol. Biol. 18 (1966) 204.
[2] M.A. Bratt and W.S. Robinson, J. Mol. Biol. 23 (1967) 1.
[3] C.D. Blair and W.S. Robinson, Virology 35 (1968) 537.
[4] M.Ya. Volkova, V.M. Zaides and V.G. Zaslavsky, Molekul. Biol. SSSR 3 (1969) 634.
[5] A.S. Spirin, N.V. Belitsina and M.A. Ajtkhozhin, Zh. Obshch. Biol. 25 (1964) 321. Translation: Federation Proc. 24 (1965) T 907.
[6] A.S. Spirin, European J. Biochem. 10 (1969) 20.
[7] V.M. Zaides, V.G. Zaslavsky, N.V. Kaverin, A.G. Bukrinskaya and M.Ya. Volkova, Molekul. Biol. SSSR 4 (1970) 607.
[8] A.S. Spirin, N.V. Belitsina and M.I. Lerman, J. Mol. Biol. 14 (1966) 611.
[9] N.V. Belitsina, L.P. Ovchinnikov, A.S. Spirin, Yu.Z. Ghendon and V.I. Chernos, Molekul. Biol. SSSR 2 (1968) 727.
[10] D.W. Kingsbury, J. Mol. Biol. 18 (1966) 195.
[11] D. Baltimore and A.S. Huang, J. Mol. Biol. 47 (1970) 263.
[12] L.P. Ovchinnikov, M.A. Ajtkhozhin, T.F. Bystrova and A.S. Spirin, Molekul. Biol. SSSR 3 (1969) 449.
[13] A.S. Huang and D. Baltimore, J. Mol. Biol. 47 (1970) 275.
[14] V.G. Zaslavsky, V.M. Zaides, M.Ya. Volkova, N.V. Kaverin and A.G. Bucrinskaya, FEBS Letters 14 (1971) 137.