A POSSIBLE REPLICATIVE FORM OF SEMLIKI FOREST VIRUS RNA

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Double stranded replicative forms of virus RNA have been described in encephalomyocarditis virus (EMC) - infected Krebs II cells (Montagnier and Sanders 1963), and in polio-infected Hela cells (Baltimore, Becker and Darnell 1964). RNA with similar sedimentation characteristics in a sucrose gradient and similarly resistant to RNA-ase has been isolated as a product from the *in vitro* incubation of the virus-specific RNA polymerase derived from polio-infected Hela cells (Baltimore 1964), and from EMC-infected Krebs II cells (Horton, Liu, Dalgarno, Martin and Work 1964). However, no studies have been reported of a similar phenomenon in primary cells or in cells infected with animal RNA viruses other than enteroviruses.

The following is a report on the early appearance in primary Chick Embryo Fibroblasts (CEF) infected with Semliki Forest Virus (SFV), an arbovirus, of RNA

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possessing some of the characteristics of the replicative forms described in the EMC-Krebs II and polio-Hela systems.

Conditions of culture of the CEF have been described (Taylor 1963). Cells were infected with SFV at a multiplicity of 10-20 PFU/cell. Actinomycin D (1γ/ml) was added at the time of infection, and the cells left overnight at 4°C. Incubation at 37°C was started the following morning. Under these conditions the latent period of the virus is 4.5 hours. Maximum virus yields are obtained at 9 hours and the maximum rate of actinomycin-resistant RNA synthesis occurs at 6 hours (Taylor 1964). Cells were labelled with tritiated adenosine for various periods during the growth cycle, following which they were scraped off the glass and homogenised in 0·1 M potassium phosphate buffer, pH 7·2, in a Dounce homogeniser. The nuclei were removed by centrifugation and the cytoplasm treated with 5% sodium dodecyl sulphate for 4 minutes at 37°C. Preparations thus obtained were analysed by sedimentation in a 5-20% sucrose gradient (0·1 M KCl, 0·01 M Tris-HCl pH 7·2, 0·001 M EDTA) after adding chick ribosomal RNA to serve as an optical density marker. Both the extraction procedure and the procedure used to prepare gradient fractions free from adsorbed nucleotides before counting will be described in detail in a subsequent publication (Dalgarno, Martin and Work - in preparation). Tritium was counted in a Packard TRI-CARB liquid scintillation counter.

Fig. 1 shows the sedimentation profile of RNA extracted from cells labelled from 3·5 to 4·5 hrs.

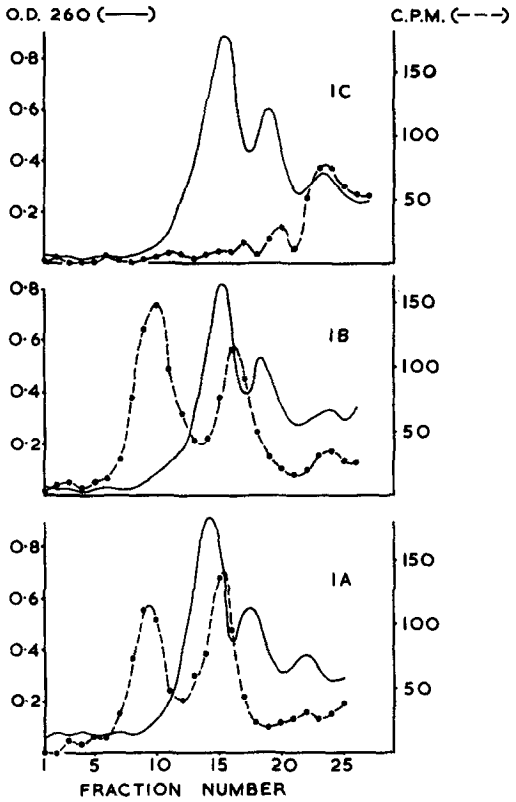

Fig.1 Sedimentation analysis of RNA extracted from the cytoplasm of SFV infected CEF labelled with H^3 adenosine for one hour at 3.5 hours (fig.1a), and 6 hours (fig.1b) after infection, and from uninfected cells labelled for 90 minutes (fig.1c). Petri dish cultures (2.5×10^7 cells/culture) infected with SFV were labelled with H^3 adenosine (30 µc); cytoplasmic extracts were treated with 5% sodium dodecyl sulphate and sedimented in a sucrose gradient (5-20% sucrose) in the SW39 rotor of the Spinco Model L ultracentrifuge for $2\frac{1}{2}$ hours at 38,000 r.p.m.

post infection (fig.1a), and from 6-7 hours post infection (fig.1b). The pattern obtained from an uninfected actinomycin D-treated control is shown in fig.1c. Two viral RNA components can be seen in material extracted from cells both early and late during the growth cycle. By comparison with chick ribosomal RNA with sedimentation coefficients of 28S and 16S, the two viral RNA components have sedimentation coefficients of approximately 36S and 24S respectively.

Material obtained from cells labelled from 4-5 hours post infection was treated with ribonuclease (C.F.Boehringer and Soehne, Mannheim) before gradient analysis. The RNA was dissolved in 0·05 M potassium phosphate buffer (pH 7·2) and treated with 3γ/ml. and 0·1γ/ml. RNA-ase for 10 minutes at 25°C. The relative resistance to RNA-ase of the more slowly sedimenting component can be seen in fig. 2. Fig.2a shows the untreated control, fig.2b and fig. 2c the effect of 3γ/ml and 0·1γ/ml. of RNA-ase respectively.

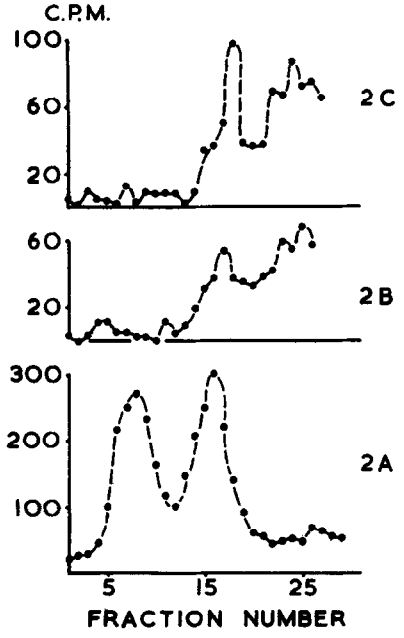

Fig.2 The effect of ribonuclease on the two RNA components extracted from SFV infected CEF labelled for 1 hour with H^3 adenosine at 4 hours after infection. RNA was treated with 3 µg/ml (fig.2b) and 0.1 ug/ml (fig.2c) ribonuclease for 10 minutes at 25°C before sedimentation analysis. Fig.2a shows the untreated control.

An attempt was made to determine whether the RNA-ase resistant form of RNA was labelled in advance of the more rapidly sedimenting component. Accordingly, at 5 hours after infection, cells were labelled for 15 minutes,

30 minutes and 60 minutes. The results of this experiment are shown in fig.3 and it can be seen that the ratio of radioactivity in the two peaks changes in favour of the rapidly sedimenting RNA with increasing labelling periods.

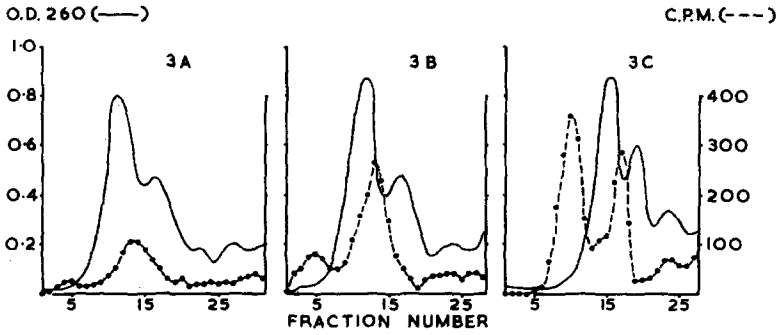

Fig.3 Sedimentation analysis of RNA extracted from SFV infected CEF labelled with H^3 adenosine at 5 hours post infection for 15 minutes (fig.3a), 30 minutes (fig.3b) and 60 minutes (fig.3c).

The 20S RNA described by Montagnier and Sanders in EMC infected-Kerbs II cells and the 16S RNA described by Baltimore, Penman and Darnell in polio infected Hela cells had properties characteristic of double stranded RNA. However, there was no clear evidence for the role of this double stranded RNA in the replication of the RNA incorporated into virus particles. The present results show that a relatively RNA-ase resistant RNA appears early in CEF infected with SFV. It may be a double stranded form similar to that described in polio-infected Hela cells and in EMC-infected Krebs II cells, and the fact that it is labelled in advance of the RNA-ase sensitive form suggests that it may play a part in its replication.

References

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