DNA Sequences in Influenza Virions

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SUMMARY

During the propagation of A (H3N2) influenza virus in chick embryos, incorporation of $^3$H-thymidine into virions takes place, whereas no such incorporation occurs with Newcastle disease virus. Incorporation of $^3$H-thymidine is a result of DNA synthesis. This virion-associated DNA is present in cores obtained after treatment of virions with bromelain.

It is well known that influenza virus reproduction is dependent on the functioning of nuclear DNA. If transcription of the cell DNA is blocked, virus replication does not start (Barry, 1964). Influenza virus nucleocapsid (ribonucleoprotein) is formed in the nucleus (Krug, 1972), the virus does not multiply in enucleated cells (Follett et al. 1974) and influenza-virus infection increases the activity of cellular DNA-dependent RNA polymerase (Borland & Mahy, 1968). Some data have been published consistent with the possibility of the presence of DNA in influenza virions. Although $^3$H-uridine-labelled virion RNA is almost completely (99.8 to 99.9%) hydrolysed by pancreatic ribonuclease (Pons, 1971), about 5% of the nucleic acid labelled with $^{32}$P in vivo or with $^{125}$I in vitro is resistant to the enzyme (Ritchey et al. 1976; Taylor et al. 1977). As long as influenza virus RNA is deprived of poly(A) sequences (Etkind & Krug, 1974), these data may be interpreted to indicate the presence of DNA in the preparations studied. This fraction appeared to be sensitive to deoxyribonuclease and was considered to be cellular DNA (Duesberg, 1968). In influenza B virions, about 14% of $^{32}$P-labelled nucleic acid was hydrolysed by deoxyribonuclease (Ritchey et al. 1976). The work described here was carried out in order to verify the possibility of the presence of DNA sequences in influenza virions.

Influenza A (H3N2) virus, strain MRC-11 was propagated, in chick embryos. The titre of this virus in the allantoic fluid was 640 haemagglutinating unit (HAU)/ml and 10$^7$ID$_{50}$/ml Newcastle disease virus (NDV), strain Beaudette, propagated in chick embryos had a titre of 256 HAU/ml and 5 x $10^7$ p.f.u./ml. After infection of chick embryos with the viruses, $^3$H-thymidine (200 $\mu$Ci/egg, sp. act. 15 Ci/mmol) or $^3$H-uridine (200 $\mu$Ci/egg, sp. act. 20 Ci/mmol) was introduced.

To prepare purified virus, allantoic fluid was clarified by low-speed centrifugation and the virus then pelleted by centrifugation in an angle rotor of a Spinco L 5-50 centrifuge at 19000 rev/min for 45 min. The pellet was resuspended in TNE buffer (tris-HCl 0.01 M, pH 7.4, NaCl 0.1 M, EDTA 0.001 M), layered on a discontinuous sucrose gradient (12–24–36–48–60%) prepared in the same buffer and centrifuged in an SW-27 rotor at 20000 rev/min for 1 h. The material at the interphase formed in 36% sucrose was diluted with TNE and pelleted by centrifugation. The pellet was resuspended in TNE, layered on a linear sucrose density gradient (15 to 40%) and centrifuged in an SW-27 rotor at 20000 rev/min for 45 min. Finally the virus was purified on 15 to 40% linear potassium tartrate density gradients (SW-27 rotor, 25000 rev/min for 5 h) and used for further experiments. The purity of virus preparations was tested in an electron microscope, its polypeptides were studied by electrophoresis in polyacrylamide gels (Zakstelskaya et al. 1975), and the absence of mycoplasmas was verified on selective medium (Timakov & Kagan, 1973).
Fig. 1. Density distribution of haemagglutination activity (■—■), \( ^3\)H-uridine radioactivity (●—●) and \( ^3\)H-thymidine radioactivity (○—○) after centrifugation of purified preparations of influenza virus (a) and NDV (b) in linear potassium tartrate density gradients (1·10 to 1·30 g/ml) prepared in TNE buffer in an SW-27 rotor of a Spinco L5-50 centrifuge at 24000 rev/min for 5 h. The virus was grown in chick embryos in the presence of \( ^3\)H-uridine (200 \( \mu \)Ci/egg) or \( ^3\)H-thymidine (200 \( \mu \)Ci/egg) and purified as described. Each part (a and b) of the figure presents the combination of results obtained in the centrifugation of \( ^3\)H-uridine and \( ^3\)H-thymidine labelled viruses. ▲—▲, Density, g/ml.
Fig. 2. Isopycnic banding of \(^{3}H\)-thymidine radioactivity of nucleic acid extracted from influenza virus cores after centrifugation in a caesium chloride gradient. Nucleic acid was dissolved in TNE buffer, solid caesium chloride was added to a density of 1.70 g/ml and the material centrifuged in an SW-50 rotor at 40000 rev/min for 48 h.

For preparation of cores purified virions were suspended in TEMB buffer (tris-HCl 0.1 M, pH 7.2, EDTA 0.001 M, \(\beta\)-mercaptoethanol 0.05 M, bromelain (Serva) 1 mg/ml) and incubated at 35 °C for 18 h (Brand & Skehel, 1972). Thereafter the suspension was centrifuged in a SW-41 rotor at 35000 rev/min for 60 min. The pellet containing subviral structures was suspended in 0.01 M-phosphate buffer (pH 7.2) containing 0.1 N-NaCl and purified in a linear sucrose density gradient (20 to 60 %) in an SW-41 rotor at 35000 rev/min for 16 h. The core preparations were studied in polyacrylamide gels (Zakstelskaya et al. 1975). Under these conditions only four polypeptides were found in the cores instead of the seven characteristic of purified virions. No haemagglutinin and neuraminidase were found in the core preparations.

To extract virion nucleic acid, purified virions were suspended in TNE buffer with 0.5 % sodium dodecyl sulphate (SDS) and the nucleic acid was extracted by phenol at pH 7.6, then once with chloroform and the preparations were precipitated three times with ethanol containing 0.2 M-sodium acetate. The preparations thus obtained had \(A_{260}:A_{230}\) ratios of 2.0 and \(A_{260}:A_{230}\) of 1.9.

Special attention was given to the purity of the viruses studied and the virus preparations were repeatedly controlled to exclude possible contamination with mycoplasmas. The purity of the virus preparations was confirmed by electron microscopy and negative results in attempts to isolate mycoplasmas in selective medium. During purification of viruses, only sterile solutions were used and all procedures were performed in the cold in maximally sterile conditions.

In purified preparations of influenza virus, grown in the presence of \(^{3}H\)-thymidine, 1 ct/min/HAU acid-insoluble radioactivity was detected whereas in NDV preparations, under the same conditions, about 0.01 ct/min/HAU was detected.

Experiments were conducted to study the absorption and elution capacities of the \(^{3}H\)-thymidine radioactivity on chick erythrocytes. Both normal chick erythrocytes (NCE) and erythrocytes treated with excess of non-labelled virus and thereafter washed (RDCE) were
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used. Virus elution was carried out with saline at 37 °C for 30 min. Initial samples of virus contained 1200 HAU and 1600 ct/min. Adsorption and elution of HAU and radioactivity were as follows: if NCE were used 80 HAU and 200 ct/min were not adsorbed but 1200 HAU and 1680 ct/min were released after elution; if RDCE was used 1200 HAU and 1500 ct/min were not adsorbed but 320 HAU and 120 ct/min eluted. It is therefore apparent that the 3H-thymidine radioactivity in influenza virus preparations adsorbs to and elutes from normal erythrocytes together with haemagglutinin.

Purified influenza virus and NDV grown in the presence of 3H-thymidine were centrifuged in linear gradients of potassium tartrate. It can be seen from Fig. 1 that the 3H-thymidine radioactivity of influenza virus is localized in the zone of the density of the virus and coincides with the position of 3H-uridine radioactivity. Similar analysis of NDV demonstrated the complete absence of 3H-thymidine radioactivity in the zone of the density of the virus.

For studying the localization of 3H-thymidine radioactivity within influenza virions purified virions labelled with 3H-thymidine were treated with various concentrations of deoxyribonuclease (10 to 100 µg/ml), which hydrolysed 80 to 98% of 3H-thymidine labelled DNA extracted from chick embryo fibroblasts. No essential decrease of radioactivity was observed thus showing that the radioactivity was located not on the surface, but inside the virions. After treatment of virions with bromelain and purification of cores 3H-thymidine radioactivity was associated with these structures. Nucleic acid was extracted from 3H-thymidine labelled cores and studied in caesium chloride gradients. As shown in Fig. 2, the radioactive component had a density of about 1.70 g/ml, which is characteristic for DNA.

The data presented in this paper allow the conclusion that DNA sequences are present in nucleic acid preparations from influenza virions. It is not possible at present to exclude a number of trivial reasons for the inclusion of DNA fragments into influenza virions. However it is interesting to note that the DNA is present in internal virus structures. Similar analysis of NDV which was grown in the same cells, demonstrated the complete absence of DNA in virus cores and in whole virus. These facts suggest that the presence of DNA sequences in virions and the mechanism of its inclusion are to a certain extent specific for influenza virus. Our investigations using 125I-DNA extracted from influenza virus (E. N. Kantorovich-Prokudina et al., unpublished observations) allow the conclusion that the DNA represents about 5 to 8% of the total nucleic acid, and additional studies of the kinetics of reassociation with this 125I-DNA indicate its host origin. Incorporation of DNA into RNA-containing viruses may not be unique for influenza virus. Cellular DNA was detected in virions of vesicular stomatitis virus (Kingsbury & Lerner, 1974) and oncorna-viruses (Deeney et al. 1976). Whether these phenomena are fortuitous or indicate a possible role for cellular DNA in virus reproduction is not known at this time.

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The D. I. Ivanovsky Institute of Virology
Gamaleya St. 16, Moscow 123098, U.S.S.R.

E. N. KANTOROVICH-PROKUDINA
N. P. SEMYONOVA
S. S. YAMNIKOVA
O. N. BEREZINA
V. M. ZHDANOV
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