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Defective Virus RNA Synthesis and Production of Incomplete Influenza Virus in Chick Embryo Cells

By D. P. NAYAK

Department of Medical Microbiology and Immunology, School of Medicine, University of California, Los Angeles, California 90024

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SUMMARY

Virus particles released from chick embryo cells infected with equine influenza virus at high multiplicity in the first 10 hr after infection contained the complete RNA component of infectious virus. Virus released later (20 to 30 hr after infection) was of von Magnus type and contained the RNAs of incomplete virus. It is concluded that the formation of von Magnus virus in chick embryo cells is due to a defective synthesis of virus RNA components and not due to a defective assembly and release of virus particles.

INTRODUCTION

Incomplete influenza virus particles are produced by cells infected at high multiplicity. This is the phenomenon of von Magnus (1951). These virus particles have the same antigenic and protein components as infectious viruses but contain a defective virus genome and are only partially infectious or non-infectious (Barry, Bromley & Davies, 1970; Duesberg, 1968; Nayak, 1969; Pons & Hirst, 1969). The largest RNA molecule (21 S) of the virus is often missing or present only in small amounts in these incomplete virus particles. The mechanism of formation of such virus particles is not clear. Two possibilities exist: either (1) all virus RNA molecules are synthesized in the cell but, because of defective maturation, are not incorporated into infectious virions, or (2) there is a defective synthesis of virus RNA's inside the cell and progeny virus particles contain a defective virus genome. Studies in HeLa cells in which influenza virus produces an abortive type of infection indicate that all the virus RNA components are synthesized in the infected cell but defective maturation and assembly cause the formation of non-infectious virus (Lerner & Hodge, 1969). On the other hand, in chick embryo (CE) cells, the multiplicity-dependent synthesis of von Magnus virus particles is possibly due to a defect in the synthesis of virus specific RNAs (Choppin & Pons, 1970; Nayak, 1969). Our studies on the replication of influenza virus RNA in CE cells infected at high multiplicity showed a gradual change in the synthesis of virus RNA. The intracellular virus-specific RNA species synthesized early in the replication cycle (4 to 8 hr post infection) were similar to the RNA species found in infectious virions. In contrast, the intracellular virus RNA species synthesized 16 hr after infection were similar to those isolated from von Magnus viruses (Nayak, 1969). These observations suggested that the multiplicity-dependent formation of incomplete virus was caused by the defective synthesis of virus RNA components in the infected cells. However, even though we and others have shown that in CE cells infected at high multiplicity there is a shift in the synthesis of intracellular virus RNA, no demonstration has been given of a corresponding shift in the RNA of progeny

von Magnus virus released at different times. The present results show that there is a shift in the RNA profile of viruses released at different times which reflects the change in the synthesis of intracellular virus RNA observed previously (Nayak, 1969).

METHODS

Viruses and cells

Equine influenza virus (MIAMI strain, EIV) and primary chick embryo cells were used in these experiments. The preparation of stock virus containing minimal amount of non-infectious virus has been described (Nayak & Baluda, 1967).

Virus infection

Primary CE monolayers were infected with infectious equine influenza virus at a high multiplicity of 20 infectious units/cell (Nayak & Baluda, 1968). The infected cells were divided into two groups. One group, immediately after infection and washing, was labelled with medium containing [^{3}H]-uridine (specific acting 26 c/m-mole) (10 μc/ml.), and the supernatant fluid was harvested 9 hr later. The cells were trypsinized to recover the virus which had not been released into the medium or which had re-adsorbed to the cell surface. Both supernatant fluids (medium and trypsin) were centrifuged at 10,000 rev./min. to remove cell debris, and the virus was adsorbed to human type 'O' red cells (Nayak & Baluda, 1967). The other group of infected cells was labelled with [^{14}C]-uridine (sp.act. 53·1 mc/m-mole 2 μc/ml.) at 16 to 25 hr after infection. The viruses in the medium and in the trypsin were then adsorbed with red cells. The red cells with viruses from both groups (0 to 8 hr and 16 to 25 hr) were mixed and the virions were eluted with receptor destroying enzymes, purified and analysed in sucrose gradients (Nayak & Baluda, 1967). RNA was isolated from purified virions and analysed in a sucrose velocity gradient.

RESULTS

Characteristics of RNA of virus released at different times after infection

The [^{3}H]-labelled RNA of the early virus had the profile of infectious virus; it was broad and heterogenous with a peak at 21 s (Fig. 1). On the other hand the [^{14}C]-labelled RNA of the late virus had the profile of von Magnus virus; heavy RNA components were either absent or present in smaller amounts, and the major RNA component in these late virions sedimented at 8·5 s. Both virus preparations also contained a small molecular weight RNA of 4 s, possibly of cellular origin.

Characteristics of the intracellular virus specific RNA synthesized at different times after infection

The next experiments were designed to see if there was a corresponding shift in the synthesis of intracellular virus specific RNA. Cells were infected and labelled 4 to 8 hr and 20 to 24 hr after infection in the presence of actinomycin-D added $\frac{1}{2}$ hr before the additions of [^{3}H]- or [^{14}C]-uridine. After labelling, cells were trypsinized and mixed and intracellular RNA was isolated and analysed in sucrose gradients (Nayak & Baluda, 1968). The profile of intracellular virus RNA reflected closely that of progeny virus RNA released at the corresponding time (Fig. 1, 2). High molecular RNA (21 s) was present only in cells labelled 4 to 8 hr after infection whereas cells labelled at 20 to 24 hr contained mostly small molecular virus RNA's.

Nature of the gradual change of RNA profile in released virions

Our earlier studies also showed in cells infected at high multiplicity the synthesis of intracellular virus RNA changed gradually with the corresponding decrease of large molecular virus RNA (Nayak, 1969). The gradual or abrupt shift from infectious to non-infectious virus RNA in the released virions was therefore tested. Cells were labelled at different times after infection and RNAs of purified virions released at different times were analysed. The RNA components of virions released at different time intervals after infection

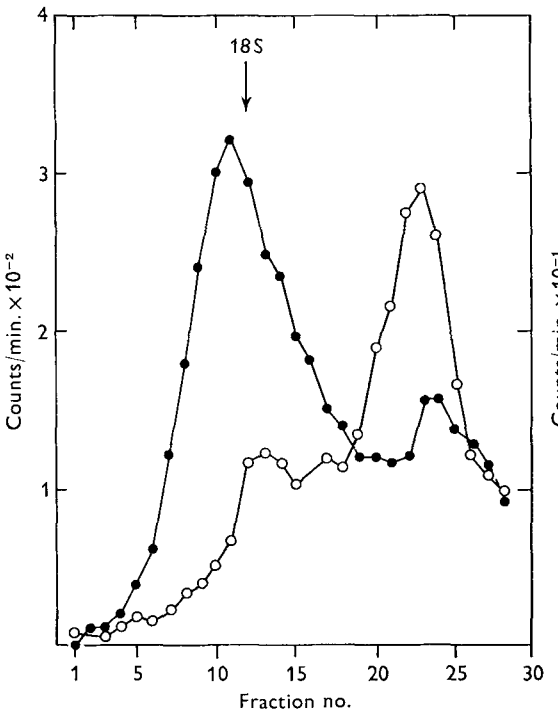

Fig. 1

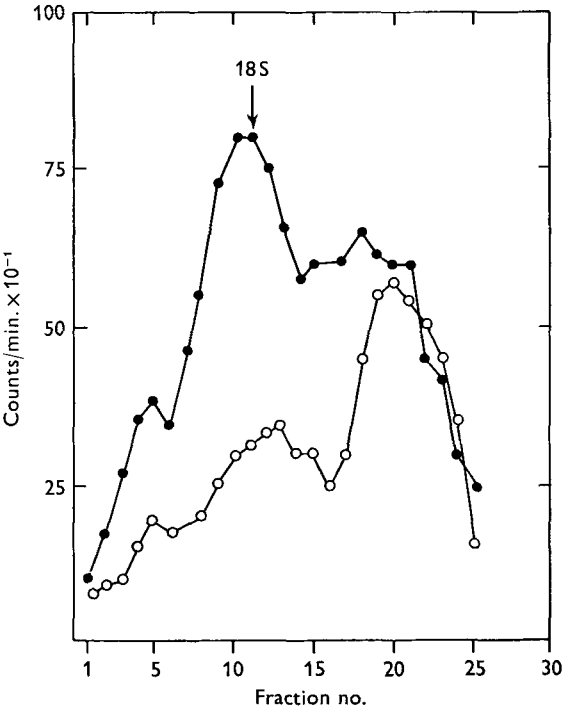

Fig. 2

Fig. 1. Sucrose velocity gradient of RNA isolated from purified influenza virions. Twenty plates (120 mm.) of chicken embryo monolayers were infected with equine influenza virus at 20 I.U./cell. Virus from cells labelled immediately after infection with [^{3}H]-uridine was harvested 9 hr after infection and mixed with virus released from cells with [^{14}C]-uridine at 16 to 25 hr after infection. The virus was purified and the RNA was isolated and analysed in a sucrose gradient with 18 s-unlabelled ribosomal RNA added as marker (20% to 5% linear gradient of sucrose containing 0·1 M NaCl, 0·01 M Tris + HCl pH 7·4, 0·001 M EDTA). The positions of marker RNA was determined from E_{260}. TCA-precipitable radioactivity, [^{3}H] (●—●) and [^{14}C] (○—○), was determined from each fraction.

Fig. 2. Sucrose velocity gradient of intracellular virus RNA synthesized at 4 to 8 hr, after infection, labelled with [^{3}H]-uridine, and at 20 to 24 hr after infection, labelled with [^{14}C]-uridine. Intracellular RNA was analysed in sucrose gradients. TCA-precipitable radioactivity: [^{3}H] (4 to 8 hr), (●—●), [^{14}C] (20 to 24 hr), (○—○).

(4 to 8 hr, 12 to 16 hr or 20 to 24 hr) revealed a gradual change (Fig. 3). The RNA profiles of virions isolated at 4 to 8 hr and 20 to 24 hr after infection corresponded to that of infectious and von Magnus virions, respectively. The RNA profile of virions labelled at 12 to

16 hr was intermediate since 21 s-labelled RNA molecules were present but few. The shift in the RNA profiles of released virions paralleled the change in the synthesis of intracellular virus specific RNA synthesis (Nayak, 1969).

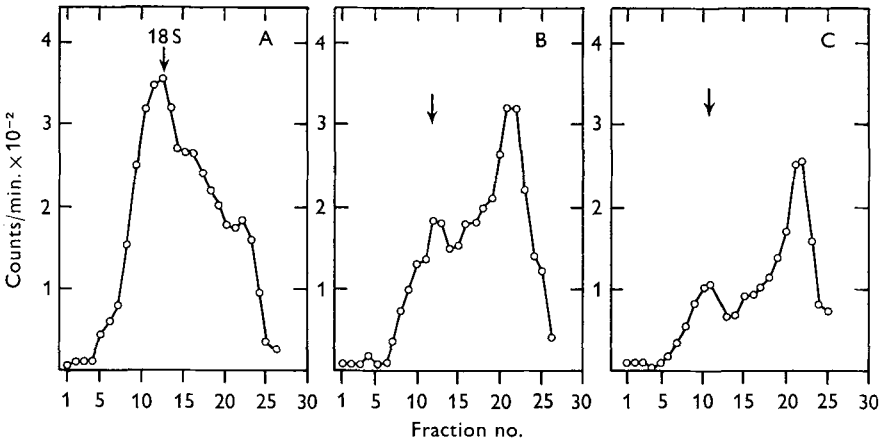

Fig. 3. Sucrose velocity gradient of RNA isolated from virus labelled with [^{3}H]-uridine released at different times after infection. (A) 4 to 8 hr, (B) 12 to 16 hr, (C) 20 to 24 hr after infection. Viruses were purified from the supernatant fluids and RNA was isolated from purified virus and analysed as in Fig. 1. Ribosomal RNA of 18 s was added as the marker.

DISCUSSION

There are inconsistencies in the published results on the nature of the RNAs of first passaged von Magnus virus produced by chick cells infected at high multiplicity. Nayak & Baluda (1967) and Pons & Hirst (1969) observed no change in the profile of first passaged von Magnus virus produced in CE cells, even though the p.f.u./HA ratio was lower in the first passaged von Magnus virions. Pons & Hirst (1969) reported that only virus particles produced after second passage lacked the 21 s RNA molecule and had the characteristic RNA profile of von Magnus virus. Barry *et al.* (1970), on the other hand, reported a change in the RNA profile even in von Magnus virus at first passage. Our results clearly indicate that the RNA profile of first passaged von Magnus virus depends entirely on the time of labelling. If the label was added early in the infectious cycle the profile of RNA of released viruses closely resembled that of infectious virus. This is the more so because most of the intracellular virus specific RNA is synthesized within 10 hr of infection (Nayak, 1969). Therefore, the label added early in the infectious cycle does not reflect the nature of the progeny virus which is usually harvested at 30 hr after infection or later. Barry *et al.* (1970) reported that a lower proportion of high molecular weight RNA occurred after longer intervals between infection and harvest of virus. However, it is not clear from their experiments whether the change in the RNA's of progeny viruses was due to change in the intracellular synthesis of virus RNA's and also due to release of virus of von Magnus type later in the infectious cycle. The mechanism by which the intracellular synthesis of large molecular virus RNA (21 s) is turned-off is not clear. Of the suggested possibilities (Choppin & Pons, 1970; Nayak, 1969) the most attractive is that a small RNA molecule interferes with the replication of a large virus RNA molecule. This has been postulated in vesicular stomatitis virus infection by Huang & Baltimore (1970). The possible role of small virus RNA molecules in the formation of von Magnus virus is unknown. Whatever mechanism may turn-off the synthesis of large

virus RNA, our experiments demonstrate some unique properties of chick cells infected with influenza virus. The same CE cells infected at high multiplicity will synthesize and release, early in the infectious cycle, virus particles containing RNA's of complete infectious virions, but later will synthesize and release virus particles of von Magnus type. These findings support therefore the earlier postulate that the formation of von Magnus virus in CE cells is due to defective synthesis of virus RNA components in the infected cell (Choppin & Pons, 1970; Nayak, 1969).

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