USE OF ACTINOMYCIN D TO UNMASK RNA SYNTHESIS INDUCED BY NEWCASTLE DISEASE VIRUS

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In several RNA-containing animal virus systems in which changes in host cell RNA metabolism resulting from virus infection have been investigated, it has been difficult to distinguish events directly related to virus synthesis from secondary effects of infection on cell metabolism (Ackermann et al., 1959; Salzman and Lockart, 1959; Martin and Work, 1961). In the case of the myxoviruses, moreover, little or no change in RNA synthesis early in the infectious cycle has been observed (Arnoff and Rafelson, 1959; Rosenbergova, 1960). If cellular RNA synthesis proceeds at near normal rates following infection, incorporation of isotopically labelled RNA precursors into a virus-induced RNA fraction is necessarily obscured.

Recent studies by Simon (1961), Reich and Franklin (1961), and Reich et al. (1961), have shown that several RNA-containing animal viruses can replicate in spite of severe impairment of DNA synthesis and DNA-dependent RNA synthesis, thereby suggesting the approach used in this study.

In the experiments to be described, the antibiotic drug, actinomycin D, was used to inhibit DNA-dependent RNA synthesis in avian cells sufficiently to reveal the cycle of RNA synthesis directed by Newcastle disease virus (NDV).

Methods: Replicate primary monolayer cultures of chick embryo fibroblasts were prepared by trypsinization of 9 day old White Leghorn em-

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bryos. Cells were grown in double strength Eagle's (1955) nutrients supplemented by 15% fetal calf serum for 48 to 72 hours before use. Cultures were infected with 200 plaque forming units (pfu) of centrifugally purified NDV (Hickman strain) per cell for 15 minutes, to synchronize the infection as much as possible. Actinomycin D was used at 10 µg/ml concentration in all experiments. The specific activity of the cytidine-H^3 employed was 1.0 curie/millimole. A semi-micro modification of the method of Schmidt and Thannhauser (1945), lipid extraction steps omitted, was used to extract RNA from cell samples. Tritium activity of the extracts was measured in a liquid scintillation spectrometer. Protein was determined by the method of Lowry (1951).

Results: Following the experimental design of Reich et al., (1961), preliminary experiments were carried out using 8 hr pretreatment of the chick embryo fibroblasts with actinomycin before infection. It was found that NDV synthesis was sharply inhibited under these conditions (Table 1). Addition of actinomycin immediately following infection inhibited virus synthesis approximately 50%. The shape of the virus growth curve under conditions of actinomycin inhibition was, however, identical to untreated controls. More extended observations of this phenomenon will be presented in a future publication.

Table 1

Effect of duration of pretreatment with 10 µg/ml actinomycin D on the yield of NDV 11 hours after infection. Each value is the extracellular (released) virus from a single cell culture.

Sample	Hemagglutinin titer/ml	Infectivity (pfu)/ml
No actinomycin	7.5	3.1×10^7
Actinomycin added t_0	5.0	1.5×10^7
Actinomycin added t_{-8}	1.0	5.4×10^6

Addition of cytidine-H^3 (1.0 μc/ml) to the cell cultures at the same time as actinomycin was added revealed the very rapid and dramatic inhibitory effect of the drug on RNA synthesis in these cells (Fig. 1). Pretreatment with actinomycin was, therefore, unnecessary for the purpose of this study.

Infection of the cells with NDV immediately before addition of actinomycin produced a clearly defined burst of RNA synthesis, as revealed by incorporation of H^3 into RNA (Fig. 1).

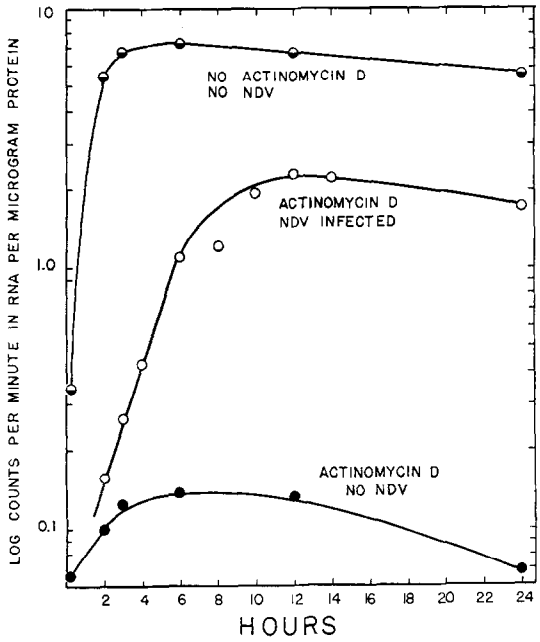

Fig. 1. Inhibition of RNA synthesis in chick embryo fibroblasts by actinomycin D (10 μg/ml); RNA synthesis in cells infected with NDV 15 minutes before addition of actinomycin D. Cytidine-H^3 (1 μc/ml) was added to all cultures nearly simultaneously. All data are from a single typical experiment employing a single cell culture for each plotted point.

The time course of synthesis of this NDV-induced RNA fraction corresponds closely to the time course of infective virus synthesis (Fig. 2), both entities becoming first detectable at 2-3 hours after infection, rising exponentially, and levelling off from 6 to 12 hours.

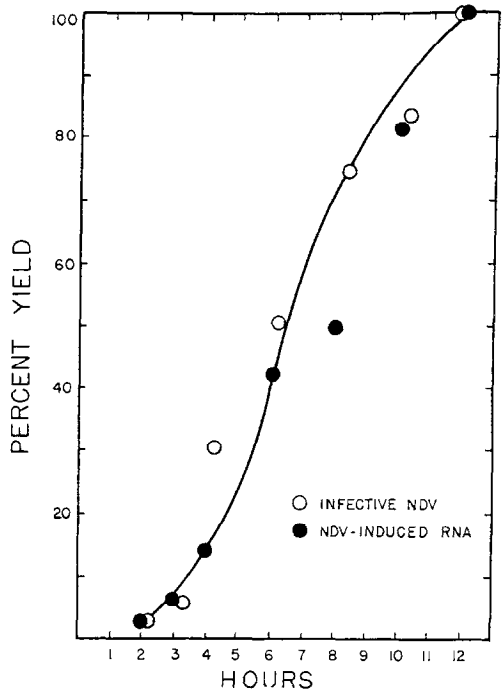

Fig. 2. The time courses of NDV-induced RNA synthesis and infective virus synthesis, taking the 12 hour point as maximum. The values for RNA represent the difference, in counts per minute/µg protein, between infected and uninfected actinomycin D treated cells. The virus growth curve was obtained in the presence of actinomycin D (10 µg/ml).

To determine how much of this RNA fraction represents material destined to be incorporated into NDV particles, the supernatants from cell cultures infected 24 hours earlier, and treated with actinomycin exactly as in the previous experiment, were subjected to density gradient centrifugation (McCrae et al., 1961). In these experiments, the level of cytidine-H^3 in the medium was raised to 5 µc/ml. Purified NDV yielded 30 counts per minute for 27 hemagglutinating units, the total extracellular virus yield of each culture. Cellular RNA from comparably treated cultures yielded slightly more than 3,000 counts per minute at 12 hours after infection, at the peak of the virus-induced RNA synthesis cycle. It is concluded, therefore, that at least part of NDV particle RNA is synthesized from the cellular nucleotide pool, and that NDV induces the synthesis of a relatively large RNA fraction, most of which never appears in progeny virus.

Discussion: It is evident that it would have been impossible to reveal the existence of the virus-induced RNA fraction described here if actinomycin D had not been employed. The significance of this fraction is enhanced by the fact of its synthesis in the face of severe impairment of normal pathways of RNA synthesis by the drug. That this RNA is not "nonsense RNA", produced by derangement of cellular control mechanisms as a result of infection by NDV, cannot be proven from the present data; but the time course of its synthesis, coinciding closely to the time course of virus assembly points strongly to the interpretation that it plays an important role in the virus replication process.

The function, then, of this RNA remains to be established. The most attractive hypothesis is that it represents templates for virus protein synthesis. The apparent short life of "messenger RNA" in other systems (Jacob and Monod, 1961) argues for the necessity of a relatively large quantity of virus-induced RNA to code virus structural protein.

It should be emphasized that the time course of synthesis of NDV particle RNA was not determined in this study. This fraction is completely obscured by the bulk of virus-induced RNA uncovered here. Other techniques (Darnell et al., 1961; Martin and Work, 1962) will be necessary to clarify this point for NDV.

It is likely that the induction by NDV of an RNA fraction much larger than is needed to supply subunits for progeny virus is not unique to NDV, and application of the actinomycin D technique used here to other RNA-containing animal viruses is to be hoped for.

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