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Messenger RNA for Interferon Production. (29850)

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Heller(1) has reported that Actinomycin D inhibits the induction of interferon production in mammalian cells even when the inducing virus used was an RNA virus whose growth would not be inhibited by actinomycin. These results have been confirmed in our laboratories(2) and elsewhere(3). These observations imply that host cell DNA contains the information for interferon production. In uninfected cells, this DNA function is repressed, but some event in virus function acts as a derepressor. The expression of this DNA as template for messenger RNA for interferon is blocked by Actinomycin D. This report is concerned with the time during which the messenger RNA for interferon is synthesized.

Experimental. The virus used, the relevant methods for interferon production and assay, and the procedures with radioactive metabolites in chick embryo tissue cultures (CE) have been described(4).

Experiments on the time course of production of interferon showed that monolayers of CE cells infected with Chickungunya virus at a multiplicity of 10 produced maximum titres of interferon at about 5 hours after infection, with the first detectable interferon being found after 2 hours. To determine whether host DNA was needed for interferon production throughout the course of infection the following type of experiment was done: Monolayers of CE cells in BME without serum were exposed to Chickungunya virus at a multiplicity of 10. At the indicated times after virus addition, actinomycin, at a concentration of 0.2 μg/ml was added. The cultures were allowed to incubate for a total of 6 hours after virus addition, and the interferon titres of the fluids determined. Controls received no actinomycin. Fig. 1 shows the results of a typical experiment of this type.

It will be seen that if actinomycin is added at any time up to 1½ hours after virus, there is no production of interferon even 5 hours later. After approximately 1½ hours of infection, the production of interferon is no longer subject to control by actinomycin, indicating that practically all the messenger RNA for interferon is made by this time.

Since a delay between time of addition of actinomycin and onset of inhibition of RNA synthesis in these experiments would necessitate a correction to the data of Fig. 1, the following experiment was performed: Actinomycin, at a concentration of 0.2 μg/ml was added to CE cells at various times, from 60 minutes before to 5 minutes after addition of H^3-uridine for 15 minutes, and the amount of incorporation into RNA determined. When actinomycin was added 60 minutes before the H^3-uridine there was an 85% inhibition in RNA synthesis (30% inhibition of protein synthesis). Even when actinomycin was added 5 minutes after the beginning of the H^3-uridine pulse, there was still a 40% inhibition. Therefore no large time correction would appear necessary for the data of Fig. 1.

Discussion. Actinomycin inhibits the synthesis of RNA(5), and also prevents migration from the nucleus to the cytoplasm of already formed RNA(6,7,8). Since by 1½ hours after infection actinomycin no longer affects the ultimate production of interferon, one can say that the messenger RNA for interferon has already been made at that time, and if interferon is made in the cyto-

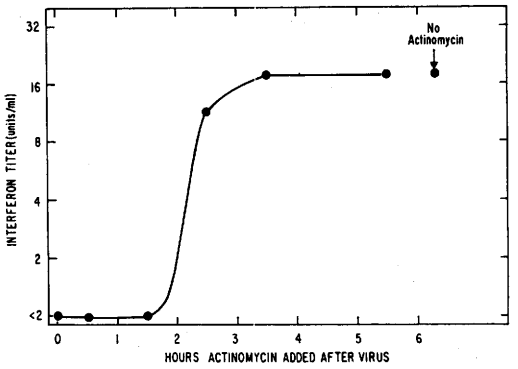

FIG. 1. Effect of addition of Actinomycin D, at different times, on production of interferon in CE cells infected with Chikungunya virus.

Monolayers of CE cells, in BME without serum, were exposed to Chikungunya virus at a multiplicity of 10. At indicated times after virus addition, actinomycin was added at a concentration of 0.2 μg/ml, and cultures allowed to incubate for a total of 6 hours after virus addition. Controls received no actinomycin.

plasm, then probably the messenger RNA is already in the cytoplasm by 1½ hours. If messenger RNA were being made continually almost from the time of addition of virus one would expect that the release from actinomycin control would be a more gradual process. The fact that an abrupt release from the control by actinomycin was found would indicate that messenger RNA for interferon became available not much before 1½ hours after infection. This is before much, if any, viral RNA has been made, but is well into the eclipse phase of the virus, where possibly other viral materials have been made. It should be mentioned that the present system was chosen because of the rapidity with which interferon is formed. What results would be found in a system where interferon is produced late during the course of infection remains to be determined.

Summary. Data have been presented which suggest that in the system of Chickungunya virus in chick embryo cells, the messenger RNA for interferon production is made available just shortly before 1½ hours after infection.

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Dengue Virus Plaque Formation in Rhesus Monkey Kidney Cultures.* (29851)

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Following Sabin's(1) adaptation of type 1 and type 2 Dengue viruses to mice, 2 and possibly 4 additional types have been isolated and identified by use of this experimental host system. Although several reports have presented evidence supporting the multiplication of mouse-adapted strains of Dengue viruses in a variety of tissue culture systems (2-9), cytopathic effects have been limited to rhesus monkey kidney(2-4), grivet monkey

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