INHIBITION OF INTERFERON ACTION BY ACTINOMYCIN

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The action of Interferon in inhibiting the growth of RNA viruses is known to be intracellular and results in an inhibition of synthesis of infectious viral RNA (1, 2). The mechanism of this inhibition remains obscure. In this communication, experiments are reported which indicate that interferon inhibits the actinomycin resistant RNA synthesis which occurs in chick embryo cells infected with Semliki Forest Virus. In the course of this investigation, evidence was obtained to suggest that the synthesis of cellular RNA is essential to the action of Interferon.

The growth of Semliki forest virus (SFV) in chick embryo fibrolasts (CEF) is susceptible to the action of chick Interferon. We have studied the mechanism of action of Interferon in this system, using preparations of Interferon purified by Zinc precipitation, and one cycle of chromatography on CM- cellulose (3). The inhibition of virus production in cells pretreated with Interferon, is paralleled by an inhibition of the actinomycin-resistant RNA synthesis occurring in virus-infected cells. The maximum rate of actinomycin-resistant RNA synthesis occurs 6-8 hours after virus infection, at which time the rate of synthesis of virus is also maximal (data to be presented elsewhere).

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The effects of pretreatment with Interferon on virus yield and on actinomycin resistant RNA synthesis are shown in Table 1. In infected cells exposed to actinomycin 44×10^{-4} μc of radioactive adenosine were incorporated per μg of RNA, whereas, in interferon treated cells, the level of incorporation of tritium was only 6×10^{-4} μc /μg RNA, which is the level found in actinomycin treated, uninfected cells. A similar effect of Interferon on actinomycin-resistant RNA synthesis in CEF cells infected with Sindbis virus has been observed by Levy and Baron (4). The inhibitory effect of Interferon on virus growth in cells not treated with actinomycin does not show as an effect on RNA synthesis since this virus does not switch off host RNA synthesis, and in the presence of Interferon, the synthesis of host RNA proceeds.

In was noted that the inhibitory effect of Interferon on virus yield was always less marked when actinomycin was subsequently added to the cells. In the experiment reported in Table 1, the yield of virus is about 6 times greater in the cells treated first with Interferon and then with actinomycin than in cells treated with Interferon only. Actinomycin can by itself result in an increased yield of virus, which may be due to the inhibitory effect actinomycin has on Interferon production during the growth of the virus (7). However, when expressed on a percentage basis, the stimulation of yield by actinomycin was always greater in Interferon treated cells than in untreated infected cells. This result indicates that actinomycin may be inhibitory to Interferon action. To test this idea, cells were treated with actinomycin before the addition of Interferon, and when the two components were added in this order, the inhibitory effect of Interferon on both virus yield and viral RNA synthesis was abolished. Data from one such experiment are shown in Table 2. The inhibitory effect of actinomycin on Interferon action is observed both with purified and crude preparations of Interferon, and with

preparations of Interferon more potent than the one used to give the data of Table 2.

Table 1

INHIBITION BY INTERFERON OF ACTINOMYCIN-RESISTANT RNA SYNTHESIS IN CEF CELLS INFECTED WITH SFV VIRUS

PRETREATMENT OF CELLS[1]		RNA SYNTHESIS 6-7 HOURS AFTER VIRUS INFECTION[2] (μc Tritium incorpd x 10^{-4}/μg RNA/hr.)		YIELD OF VIRUS[3] (p.f.u. / plate)
5 HOURS $37^{\circ}C$	OVERNIGHT $4^{\circ}C$	UNINFECTED CELLS	INFECTED CELLS	
BUFFER	BUFFER	59	57	5.3×10^7
BUFFER	ACTINOMYCIN[4]	7	44	1.1×10^8
INTERFERON	BUFFER	51	54	4.1×10^5
INTERFERON	ACTINOMYCIN[4]	7	6	2.6×10^6

1. Incomplete monolayers of CEF cells were treated with Interferon or buffer and then with Actinomycin or buffer for the times and temperatures indicated in the Table. Where cells were infected, virus was allowed to adsorb from 0.3 ml. at $4^{\circ}C$. for 1 hour before addition of Actinomycin and incubation at $4^{\circ}C$. Multiplicity of infection = 10:1. After overnight incubation, the temperature of all the plates was raised to $37^{\circ}C$ (this point was taken as the zero time point of virus infection). One hour later the cells were washed free of virus, actinomycin, and Interferon, and fresh medium added (Geys salt solution containing 0.25% lactalbumen hydrolysate, 0.1% peptone and 2.5% calf serum).

2. 10 μc.tritated adenosine were added to each plate (2 plates each point) 6 hours after virus infection. At 7 hours cells were washed free of unadsorbed tritium, freed from the glass by freezing and thawing, the RNA was isolated by a modified Schmidt Thanhauseer procedure (5) and estimated by the orcinol method (6). Tritium was estimated in a TRI-CARB scintillation counter.

3. At 7 hours after virus infection, cells were frozen and thawed to liberate virus which was titrated as plaques on CEF monolayers (2 plates were used for each point).

4. 1 μg/ml of Actinomycin D.

The primary action of actinomycin is to inhibit RNA synthesis on a DNA template (8) and the results reported here therefore suggest that DNA dependent RNA synthesis is essential to the action of

Table 2

INHIBITION OF INTERFERON ACTION BY ACTINOMYCIN

PRETREATMENT OF CELLS[1]		RNA SYNTHESIS 6-7 HOURS[2] AFTER VIRUS INFECTION (μc Tritium incorpd x 10^{-4}/μg RNA/hr.)		YIELD OF VIRUS[2] (p.f.u. / plate)
2 hours 37°C then overnight 4°C	2 hours 37°C	UNINFECTED CELLS	INFECTED CELLS	
BUFFER	BUFFER	51	45	1.1×10^8
ACTINOMYCIN[3]	BUFFER	7	35	7.7×10^7
BUFFER	INTERFERON	58	41	1.6×10^7
ACTINOMYCIN[3]	INTERFERON	7	41	7.3×10^7

1. Incomplete monolayers of CEF were treated with actinomycin or buffer and then Interferon or buffer, as indicated. Where cells were infected, virus was added after the incubation with Interferon, and allowed to adsorb for 1 hour at 37°C before washing off and replacing with fresh medium. Multiplicity of infection 10:1.

2. RNA synthesis and virus yield measured as indicated for Table 1.

3. 1 μg/ml Actinomycin D.

Interferon in the system we have used. The synthesis of the RNA could be important per se, but it is possible that it is only important indirectly in its capacity to control the synthesis of protein. Preliminary experiments with p-fluoro-phenyl alanine indicate that this inhibitor does reverse to some degree the inhibitory effect of Interferon on virus growth, suggesting that protein synthesis is indeed essential for Interferon action. An attractive hypothesis is that Interferon stimulates or induces cells to form a specific protein such as ribonuclease which serves to inhibit the replication of SFV RNA in these cells.

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