Interferon Production and Viral Ribonucleic Acid Synthesis in Chick Embryo Cells

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Although virus-induced interferon formation has been studied for some 10 years, it has not been possible to establish whether the inducer is the viral nucleic acid, protein, or some other molecule. However, the available evidence suggests that it is the viral nucleic acid (1). If this is so, then its replication may be an essential part of the induction process when infective viruses are used in interferon production, and possibly also when non-infective viruses are used. The latter, inactivated under carefully controlled conditions, may still stimulate limited nucleic acid synthesis, insufficient for the formation of complete virus, but possibly sufficient to participate in the interferon induction process.

Viral RNA synthesis often represents too small a proportion of the total cellular RNA synthesis to be readily detected unless cellular RNA synthesis is depressed in some way. Actinomycin has been widely used for this purpose since it inhibits DNA-dependent RNA synthesis, but does not affect the RNA-directed synthesis of RNA involved in the replication of many RNA viruses.

This report describes an investigation of the induction of interferon formation and concomitant RNA synthesis using an actinomycin-resistant RNA virus. The rationale behind the experiments was to show initially that viral RNA synthesis could be detected in actinomycin-treated cells using radio-isotopes; to show secondly that the virus could produce interferon under conditions where it could not multiply; and finally to determine whether, under these conditions, the virus caused an increase in the rate of RNA synthesis in actinomycin-treated cells. No interferon was produced in this last experiment since actinomycin-treated cells were used. It was assumed that any stage in the interferon induction process involving viral RNA replication occurs before the actinomycin-sensitive stage. We conclude that virus-induced RNA synthesis is not necessary for interferon production.

Semliki Forest virus was used because it multiplies normally in actinomycin-treated cells, causing a stimulation in the rate of RNA synthesis (2). Infection at 37° leads to interferon formation as well as to virus production (2), but infection at 42° to interferon production only (3).

Using methods previously described (4), the virus-induced stimulation of RNA synthesis was readily detected in actinomycin-treated cells (Fig. 1a). The amount of radioactivity was measured in both the trichloracetic (TCA)-insoluble fraction (Fig. 1a) and the TCA-soluble fraction (Fig. 1b). The radioactivity of the former was due to RNA, while that of the latter was due to the precursor nucleoside and nucleoside phosphates. The reduction in the radioactivity of the TCA-soluble material about 1 hr after infection was a consequence of actinomycin inhibition of cellular RNA synthesis (5), while the increase about 5 hr after infection was probably a consequence of the increased rate of RNA synthesis in infected cells compared with controls. The stimulation of the rate of RNA synthesis preceded the most rapid rate
of virus production by about 2 hr, suggesting that virus-induced RNA accumulated within the cells. This virus-induced RNA was shown by sucrose gradient centrifugation to contain several RNA species, which are now under investigation.

Fig. 1. The effect of infection with purified Semliki Forest virus (8) of actinomycin-treated chick embryo cells on (a) the rate of RNA synthesis, and (b) the radioactivity extracted by cold TCA. Triplicate cultures were pretreated with actinomycin (0.5 μg/ml.) for 4 hr at 37°, before infection with Semliki Forest virus (5 p.f.u./cell) for 1 hr at 37°, washing, and pulsing with [14C] uridine (0.5 μc/culture) for 20 min. at 20 min. intervals. (Control, ■—■; virus infected, ○—○; virus yield, plotted arithmetically, □—□.)

Fig. 2. The production of virus (■—■) and interferon (○—○) when chick embryo cells were incubated at (a) 37°, or (b) 42° after infection with Semliki Forest virus (5 p.f.u./cell) for 1 hr at 37°.

Fig. 3. The effect of infection with purified Semliki Forest virus on the rate of RNA synthesis in actinomycin-pretreated cells at 42°. Cells were pretreated and infected as Fig. 1 before pulsing with [3H]uridine (10 μc/culture) for 30 min. at hourly intervals. (Control, ■—■; virus infected, ○—○.)
Measurement of interferon production during the multiplication of Semliki Forest virus at 37° showed that it was produced late in the growth cycle (Fig. 2a, cf. (2)). However, when the cells were incubated at 42°, after infection at 37°, no virus was produced, but after a delay of 2 hr, interferon was formed at a linear rate until about 8 hr after infection (Fig. 2b). When actinomycin-treated cells were infected at 37°, and then incubated at 42°, there was no detectable increase in the rate of RNA synthesis in infected cells (Fig. 3). It was concluded that there was no viral RNA synthesis under these conditions, and that viral RNA synthesis was not necessary for interferon formation.

We have also used fowl plague virus inactivated by the ethylene-iminoquinone Bayer A 139, since it has been reported that the inactivated virus was still able to induce viral RNA synthesis and induce interference (6, 7). Thus it was possible that, in this case, viral RNA synthesis was essential for interferon production. Fowl plague virus was inactivated as previously described, and the product obtained after 8 and 12 hr inactivation tested for its ability to induce interferon formation and also for interference. No interferon could be detected 48 hr after infection (Plaque Depressing Dose 50 < 2) when cells were treated with virus at multiplicities of 10, 2 and 0-4 p.f.u./cell. The inactivated virus did not interfere with a Chikungunya virus challenge (PDD 50 < 10), but did interfere with a fowl plague virus challenge (PDD 50 = 80), suggesting that the interference observed previously (5) was due to destruction of the cell receptors for myxovirus attachment, receptors which do not affect the attachment of arboviruses.

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References


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