Inhibition of Host-specific DNA and RNA Synthesis in KB Cells Following Infection with Frog Virus 3

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(Accepted 28 April 1971)

SUMMARY

In KB cells infected with frog virus 3 at 37°, a non-permissive temperature for frog virus 3, there was a marked inhibition of host-cell RNA and DNA synthesis shortly after infection. The inhibition, which was as effective with gamma-irradiated or heat-inactivated virus as with live frog virus 3 was dependent upon the multiplicity of infection with frog virus 3. The effect of frog virus 3 could not be abolished by chemical inhibitors of protein and RNA synthesis. These findings suggested that a component of the virus particle was responsible for the inhibition of host-cell RNA and DNA synthesis.

INTRODUCTION

Frog virus 3 (FV 3) is a deoxyribovirus which replicates in the cytoplasm of amphibian and mammalian cells at low temperature (Granoff, Came & Breeze, 1966). FV 3 infection rapidly inhibits host-cell DNA, RNA and protein synthesis at permissive and non-permissive temperatures (Maes & Granoff, 1967; Kucera & Granoff, 1968; McAuslan & Smith, 1968; Granoff, 1969; Guir, Dole & Kirn, 1970a). Moreover it has been shown that in KB cells infected at 37° with FV 3 and vaccinia virus that there was an inhibition of vaccinia DNA synthesis (Aubertin & Kirn, 1969; Aubertin, Guir & Kirn, 1970 a). The mechanisms by which FV 3 in particular and DNA viruses in general, inhibit macromolecular synthesis are unknown. It was of special interest to study the high inhibitory effect of FV 3 on KB cells with regard to the absence of virus products synthesized at 37° in these cells (Guir, Braunwald & Kirn, 1970b). We have shown previously that in KB cells infected at 37° with FV 3 there was a marked inhibition of host-cell RNA and DNA synthesis shortly after infection. Here we report further studies on this inhibition. In particular, studies have been made to investigate the requirement for a functional virus genome and de novo synthesis of protein or RNA for inhibition of macromolecular synthesis in cells infected with FV 3.

METHODS

Tissue culture and media. KB cells were grown in lactalbumin yeast extract-Earle's salt + 5% calf serum. For the experiments, Eagle's minimal essential medium supplemented with 5% dialysed calf serum was used as maintenance medium.

Frog virus was kindly provided by Dr A. Granoff. Propagation, titration of FV 3 in tissue culture and procedures used for crude or purified preparations of FV 3 have been described (Aubertin et al. 1970 a). All experiments were performed with unpurified preparations except
those for which γ-inactivated virus was used. Crude cell extracts were prepared from uninfected cells in a similar manner as unpurified virus preparations.

*Inactivation of virus.* Virus inactivated by γ rays was prepared according to the technique described by Decker, Guir & Kirn (1969). Virus was inactivated by heating at 56° for 60 min. (Aubertin et al. 1970a).

*Infection of KB cells.* Monolayers of KB cells received either FV 3 or crude cell extracts for 45 min. at 37° and then 5 ml. of labelled medium was added to each bottle. Since crude cell extracts did not show any inhibitory effect (Guir et al. 1970b), in most experiments uninfected cells received only medium. In all experiments the KB cells were incubated at 37°.

**RNA and DNA synthesis in uninfected and in FV 3-infected cells.** Procedures for the measurement of DNA and RNA synthesis have already been described (Guir et al. 1970b). [3H]thymidine (specific activity 18 c/m-mole) and [3H]uridine (specific activity 17 c/m-mole) were used at a concentration of 0.2 μc/ml. The efficiency of the counting procedure was 30%.

**Chemicals.** Puromycin was purchased from Nutritional Biochemicals Corporation. Actinomycin was made available through the courtesy of Merck Sharp and Dohme. [3H]-thymidine and [3H]uridine were purchased from C.E.A. (Saclay).

**RESULTS**

*Influence of multiplicity of infection on host cell DNA and RNA synthesis*

KB cells were infected with 20, 10, 5, 2.5, 1, 0.5 and 0.1 p.f.u./cell and control cells received an uninfected cell extract. After an adsorption period of 45 min. fresh growth medium labelled with [3H]thymidine or [3H]uridine was added to the cells. At 2, 4 and 6 hr after infection the amount of [3H]thymidine and [3H]uridine incorporated into DNA and RNA respectively was measured and the results were expressed as percent inhibition against multiplicity of infection on host cell DNA and RNA synthesis (Fig. 1a and 1b). The rate of inhibition of cellular DNA synthesis by FV 3 clearly depended on the virus input (Fig. 1a). The influence of multiplicity of FV 3 infection on host-cell RNA synthesis is shown in Fig. 1b. These results show that at high multiplicities, the inhibition of incorporation of [3H]uridine into RNA was not significantly changed for 20, 10 or 5 p.f.u./cell. At low multiplicity of infection, the rate of inhibition of cellular RNA synthesis is greater than the inhibition of cellular DNA synthesis. The maximum inhibition of host-cell RNA synthesis required only a low multiplicity of infection (1 p.f.u./cell). By contrast, more than 5 p.f.u./cell were necessary to obtain maximum inhibition of cellular DNA synthesis. In all further experiments 10 p.f.u./cell were used.

*Host cell DNA synthesis in infected cells treated with inhibitors of protein and RNA synthesis*

The following experiments were designed to test whether protein or RNA syntheses were required for the inhibition of cellular DNA synthesis. The rate of [3H]thymidine incorporation into cellular DNA in cells infected with 10 p.f.u./cell of FV 3 declined much faster than in uninfected cells treated with puromycin or actinomycin D and therefore made it possible to study some specific virus effects in the presence of these inhibitors. The addition of puromycin (20 μg./ml) or actinomycin D (4 μg./ml) at the time of infection caused a rapid inhibition of cellular DNA synthesis (Fig. 2). The effect of FV 3 on host-cell DNA synthesis was higher in treated cells than in untreated cells. These results do not rule out the possibility that protein synthesis involved in the inhibition of DNA synthesis occurs just before the puromycin becomes fully active. In further experiments cells were pre-treated with puromycin
Host DNA and RNA inhibition by FV 3

Fig. 1. Relationship between dose of FV 3 and % inhibition of cellular DNA and RNA synthesis in KB cells. Cell cultures were infected with different multiplicities (p.f.u./cell) of FV 3. The results are expressed as % inhibition against m.o.i: ■, uninfected cell extract; ●, 0.1 p.f.u./cell; △, 0.25 p.f.u./cell; □, 0.5 p.f.u./cell; ○, 1 p.f.u./cell; △, 2.5 p.f.u./cell; ■, 5 p.f.u./cell; ●, 10 p.f.u./cell; ▲, 20 p.f.u./cell. a, effect of multiplicity of FV 3 infection on the inhibition of cellular DNA synthesis; b, effect of multiplicity of FV 3 infection on the inhibition of cellular RNA synthesis.

Fig. 2. Effect of FV 3 on cellular DNA synthesis in KB cells treated with inhibitors of RNA and protein synthesis at the time of infection. ○—○, control; ●—● FV 3 (10 p.f.u./cell); □—□ and △—△, controls with actinomycin D (4 μg/ml) and with puromycin (20 μg/ml), respectively; ■—■ and ▲—▲, FV 3 with actinomycin D and puromycin, respectively at the time of infection.
at a concentration of 20 μg./ml. 30 min. before infection producing an 83 % inhibition of protein synthesis within 30 min. Similar results were obtained in cells treated with the drug at the time of infection (Fig. 3). If the cells were pre-treated with puromycin at a concentration of 40 μg./ml. which caused a 91 % inhibition of protein synthesis within 30 min., the rate of [3H]thymidine incorporation into the cellular DNA in normal cells declined much faster than in untreated FV 3-infected cells. Nevertheless, the effect of FV 3 on cellular DNA synthesis in the presence of this concentration of puromycin was higher than that caused by the drug alone. These experiments demonstrate that after FV 3 infection, the synthesis of protein and RNA is not required for the virus inhibition of cell DNA synthesis.

![Graph](image)

**Fig. 3.** Effect of FV 3 on cellular DNA synthesis in KB cells pre-treated with puromycin 30 min. before infection. ○—○, control; ●—●, FV 3 (10 p.f.u./cell); △—△, control with puromycin (20 μg./ml.); ▲—▲, FV 3 with puromycin.

**Host-cell RNA synthesis in infected cells treated with an inhibitor of protein synthesis**

The time course of inhibition of RNA synthesis with the high multiplicity used here did not differ significantly from that observed for DNA synthesis. It was of considerable interest to see if protein synthesis was a prerequisite for the inhibitory effect of FV 3 infection on RNA synthesis of the host cell. Similar experiments to those performed for the inhibition of host-cell DNA synthesis were designed to test this possibility. The rate of [3H]uridine incorporation into host-cell RNA in FV 3-infected cells decreased more rapidly than in uninfected cells treated with puromycin (20 μg./ml.) (Fig. 4). Addition of puromycin at the time of infection caused rapid inhibition of RNA synthesis. This last inhibition was higher than that caused by the virus without puromycin. The results obtained by pre-treating the cells for 30 min. before infection with puromycin at a concentration of 80 μg./ml. which caused a 95 % inhibition of protein synthesis within 30 min. were similar to those observed with cells treated with the drug at the time of infection (Fig. 5). These experiments demonstrate that after FV 3 infection the synthesis of proteins is not required for the virus inhibition of host-cell RNA synthesis.
Host DNA and RNA inhibition by FV 3

Fig. 4. Effect of FV 3 on cellular RNA synthesis in KB cells treated with puromycin at the time of infection. ○—○ control; △—△ control with puromycin (20 μg./ml.); •—•, FV 3 (10 p.f.u./cell); ▲—▲, FV 3 with puromycin.

Fig. 5. Effect of FV 3 on cellular RNA synthesis in KB cells pre-treated with puromycin 30 min. before infection. ○—○, control; △—△, control with puromycin (80 μg./ml.); •—•, FV 3 (10 p.f.u./cell); ▲—▲, FV 3 with puromycin.
Effect of inactivated FV 3 on host-cell DNA and RNA synthesis

Further studies were made into the mechanism of the inhibitory activity of FV 3 on cell RNA and DNA synthesis by investigating the effect of gamma irradiation and heat on the ability of the FV 3 to inhibit host-cell RNA and DNA synthesis. After infection of the cells with FV 3 inactivated by heating at 56° for 1 hr, the ability to inhibit cellular RNA and DNA synthesis was not found to be significantly impaired. Under the conditions described, infective virus was not detectable after heat treatment. The same rates of inhibition of RNA synthesis were observed with live or heated FV 3 (Fig. 6). From the results recorded in Table 1, the inhibition of RNA and DNA synthesis 4 hr after infection appeared to be equally sensitive to heat-inactivated virus. Further studies showed that heating the virus for 2 hr at 56° did not greatly impair the inhibitory property of FV 3.

![Graph showing rate of RNA synthesis](image)

*Fig. 6. Rate of cellular RNA synthesis in KB cells infected with heat-inactivated frog virus. O—O, control; □—□, live FV 3 (10 p.f.u./cell); △—△, heat-inactivated FV 3 (1 hr at 56°).*

<table>
<thead>
<tr>
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<th>Inhibition of [3H]thymidine incorporation (%)</th>
<th>Inhibition of [3H]uridine incorporation (%)</th>
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<tbody>
<tr>
<td>Live FV 3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Heat-inactivated FV 3</td>
<td>89</td>
<td>88</td>
</tr>
</tbody>
</table>

[3H]uridine and [3H]thymidine were added at the time of infection. Samples were removed 4 hr after infection with 10 p.f.u./cell of live FV 3 or FV 3 inactivated by heating for 60 min. at 56°. The results were expressed on live FV 3, as a 100% maximal inhibitory effect on host-cell RNA and DNA synthesis.

As shown in Table 2 inactivation of FV 3 for 90 hr (27 × 10^5 r.) by gamma rays had no effect on the ability to inhibit cellular DNA or RNA synthesis at an irradiation dose which completely inhibited virus infectivity (Aubertin, Decker & Kirn, 1970b). In these experiments purified virus was used.
Host DNA and RNA inhibition by FV 3

[3H]uridine and [3H]thymidine penetration in FV 3-infected cells

The effect of FV 3 on the ability of KB cells to take up DNA and RNA precursors from the medium was investigated. In particular, a possible cause for the decrease in uridine or thymidine incorporation after FV 3 infection may be the failure of [3H]uridine or [3H]thymidine to penetrate the cell. This possibility was investigated as follows. The cells infected with 10 p.f.u./cell of FV 3 were incubated for 4 hr with [3H]uridine (0.4 μc/ml.) and [3H]thymidine (0.6 μc/ml.). After the cells were washed rapidly three times with cold phosphate-buffered saline the trichloroacetic acid soluble radioactivities were determined. Results obtained show that the amount of the trichloroacetic acid soluble radioactivity for [3H]thymidine was virtually the same in uninfected cells as in FV 3-infected cells (Table 3). For [3H]uridine the amount of radioactivity was twice as high in infected as in uninfected cells. Therefore, it can be concluded that in FV 3-infected KB cells the permeability to low molecular weight precursors was not decreased by infection.

Table 2. Rate of incorporation of [3H]uridine and [3H]thymidine respectively into RNA and DNA of KB cells infected with either live or γ-inactivated purified FV 3

<table>
<thead>
<tr>
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<th>[3H]uridine counts/min.</th>
<th>[3H]thymidine counts/min.</th>
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<tbody>
<tr>
<td>Control</td>
<td>50,558</td>
<td>28,202</td>
</tr>
<tr>
<td>Purified live FV 3</td>
<td>10,044</td>
<td>9,599</td>
</tr>
<tr>
<td>Purified γ-inactivated FV 3</td>
<td>10,917</td>
<td>10,174</td>
</tr>
</tbody>
</table>

The cells were infected with 4 p.f.u./cell of live FV 3 and with a dose of γ-inactivated virus corresponding to 4 p.f.u./cell before irradiation. Radioactivity was determined 4 hr after infection.

Table 3. Incorporation of [3H]thymidine and [3H]uridine for 4 hr into the acid-soluble fraction of KB cells infected with 10 p.f.u. of FV 3

<table>
<thead>
<tr>
<th></th>
<th>[3H]thymidine counts/min.</th>
<th>[3H]uridine counts/min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,205</td>
<td>22,599</td>
</tr>
<tr>
<td>FV 3 (10 p.f.u./cell)</td>
<td>1,247</td>
<td>44,339</td>
</tr>
</tbody>
</table>

The radioactivity of the acid-soluble fraction (5 ml.) was determined on 0.2 ml. samples.

DISCUSSION

These experiments afford some information about the nature of the products responsible for the inhibition of host-cell RNA and DNA synthesis and the mechanism of action of this inhibition by FV 3. Our results clearly demonstrate that the effect of FV 3 on DNA and RNA synthesis is dependent on the multiplicity of infection. The 50% inhibition of host-cell nucleic acid synthesis produced with 0.1 p.f.u./cell suggests that non-infective particles are also effective inhibitors. From the data of Smith & McAuslan (1969) it is clear that in purified FV 3 stocks there was a large amount of non-infective particles. Electron microscopic studies are in progress to determine the exact p.f.u./particle ratio. The retention of the ability to block host-cell macromolecular biosynthesis by irradiated or heated virus indicates that a heat-stable component of the virus is responsible for the effect on host-cell RNA and DNA synthesis. Our results confirm previous observations by Gravell (1969) who showed that u.v.-inactivated virus inhibited cellular RNA and DNA synthesis in F.H.M. cells. Heat-inactivated FV 3 was also capable of suppressing synthesis of host cell macromolecules. Similar results concerning the ability to block cellular DNA synthesis were found
by Jungwirth & Launer (1968) with heat-inactivated and u.v.-irradiated WR vaccinia virus; similar results have been described by Newton (1968) using u.v.-inactivated herpes simplex virus.

Thus, these findings suggest the presence of a thermostable inhibitor in the virus particle. Such an inhibitor could depress host-acid nucleic synthesis either by interacting directly with host template or by releasing some cellular enzymes. In this last connexion it should be pointed out that incubation of FV 3-infected cells at 37° for 3 or 6 hr does not cause a disturbance in virus DNA synthesis when the temperature is subsequently lowered to 26° (Aubertin et al. 1970a). The inhibition initiated by some component of the virus, or the molecules released by it, must be able to differentiate between virus and cellular DNA with respect to both replication and transcription.

The precise mechanism of inhibition of RNA and DNA synthesis by FV 3 has not been determined. However, our results show that, after infection, de novo synthesis of protein is not necessary for the inhibition of host cell nucleic acid synthesis. These results confirm those of Gravell (1969) using F.H.M. cells at 26° R. Vilaginez & B. R. McAuslan (personal communication) have also shown that FV 3 blocks initiation of poxvirus DNA synthesis under conditions where DNA synthesis was insensitive to inhibition by cycloheximide. On the contrary, with herpes virus, the results concerning this point are contradictory (Roizman & Spear 1969).

At the moment it is not clear whether the inhibition of RNA and DNA synthesis is mediated by the same mechanism. In poxvirus infection, cellular DNA synthesis is abolished before the RNA synthesis is depressed, and therefore the two inhibitions are not closely related (Becker & Joklik, 1964; Salzman, Shatkin & Sebring, 1964), whereas for herpes infection (Roizman, 1969) and FV 3 infection, host RNA and DNA syntheses are simultaneously inhibited during the first hr after infection. The results of Costanzo et al. (1970) suggest that FV 3 inhibits the activity of high ionic strength stimulated nuclear RNA polymerase. The data of R. Vilaginez and B. R. McAuslan (personal communication) are consistent with the idea that some structural component of the virus combines with uncoated poxvirus DNA and prevents transcription. Therefore, it seems probable that FV 3 acts directly on heterologous DNA transcription by a mechanism that would block poxvirus or cellular RNA polymerase activity. It is not clear whether the decrease in activity is due to inactivation of enzymes or of templates. On the other hand, the mechanism of inhibition of host DNA synthesis is not easy to understand. It seems, however, that this inhibition is not dependent upon protein synthesis. It has been shown with adenovirus that the fibre protein inhibits by binding to DNA (Levine & Ginsberg, 1967, 1968). Similar material may be associated with FV 3 particles. It is noteworthy that in a preliminary work we have shown that FV 3 inhibits Sindbis virus RNA synthesis under conditions when RNA virus synthesis was insensitive to inhibition by puromycin. It follows that FV 3 is also able to depress RNA synthesis independently of a DNA primer, perhaps by acting directly on virus RNA replicase. We are trying to elucidate some aspects of the inhibition caused by FV 3 infection in continuing to study the effect of FV 3 on host-cell macromolecule-synthesis and on virus replication of superinfecting virus in FV 3-infected KB cells. This last system affords a good opportunity to test hypotheses on this subject.

This work was supported in part by D.R.M.E. (contract no. 69.34.635.00.480.75.01), in part by Fondation pour la Recherche Médicale Française and in part by a grant from C.E.A. (Saclay). We wish to thank Miss Madeleine Klein, Miss Yolande Pagniez and Mrs Cécile Travo for skilful technical assistance.
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(Received 8 March 1971)