Effect of U.v.-irradiated Vesicular Stomatitis Virus on Nucleic Acid Synthesis in Chick Embryo Cells

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(Accepted 24 April 1970)

SUMMARY

When monolayer cultures of chick embryo cells were infected with the NEW JERSEY serotype of vesicular stomatitis virus at high input multiplicities, the synthesis of cellular RNA was rapidly inhibited. The stimulation of cellular DNA synthesis observed in control cells following medium replacement was also inhibited in the infected cells. These activities of vesicular stomatitis virus on the synthesis of cellular nucleic acids were quite resistant to u.v. irradiation, although no virus RNA synthesis was detected in the cells infected with u.v.-irradiated vesicular stomatitis virus. No stimulation of degradation of cellular nucleic acids was observed in cells treated with irradiated vesicular stomatitis virus.

INTRODUCTION

There are many reports on animal viruses which induce rapid and profound inhibition of macromolecular synthesis in infected cells (Martin & Kerr, 1968). These shut-off phenomena may provide a clue to regulatory mechanisms governing macromolecular syntheses in animal cells. Such studies are usually complicated by the multiplication of virus in the infected cells, and it is hard to distinguish effects caused by the initial, infecting particles, from those due to virus products. If non-replicating particles shut off the synthesis of cellular macromolecules then such a system would be more suitable for these studies.

Huang & Wagner (1965) reported that RNA synthesis in Krebs-2 cells was inhibited rapidly by infection with u.v.-irradiated vesicular stomatitis virus (u.v.-VSV). We have investigated the changes in macromolecular synthesis in chick embryo cells due to infection with u.v.-VSV and this paper is concerned with the nature of the inhibition by u.v.-VSV of the synthesis of cellular nucleic acids.

METHODS

Media. The complete medium was composed of Eagle’s minimal essential medium with 5 % calf serum and antibiotics supplemented with sodium bicarbonate. Phosphate-buffered saline (PBS) was free of Ca^{2+} and Mg^{2+} ions. Radioactive compounds were: [2-^{14}C]-thymidine (0·1 mc./0·1 mg., New England Nuclear Corp., Boston), [2-^{14}C]uridine (0·1 mc./0·5 mg., New England Nuclear Corp.), and [^{14}C]amino acid ([^{14}C]protein hydrolysate, Radiochemical Centre, Amersham, Buckinghamshire). Actinomycin D was a gift from the Merck, Sharp and Dohme Research Laboratory (Rahway).
Viruses. Vesicular stomatitis virus New Jersey serotype, was supplied by Dr Y. Kawade, Institute for Virus Research, Kyoto University, Kyoto. It was propagated at low multiplicity of infection in chick embryo cell monolayers, and vesicular stomatitis virus particles were partially purified according to the method of Huang, Greenawalt & Wagner (1966). The medium containing VSV was centrifuged at 2000 rev./min. for 10 min., and the supernatant was recentrifuged at 20,000 rev./min. for 60 min. The pellet was resuspended in PBS and dispersed by sonic treatment. For u.v. irradiation, 3 to 5 ml. of VSV suspension containing 3 to $8 \times 10^6$ p.f.u./ml., were shaken in a 10 cm. Petri dish and irradiated by a Toshiba germicidal lamp (15 w, 90 v) at a distance of 30 cm. The infectivity fell to $10^{-5}$ to $10^{-6}$ of the original level after irradiation for 30 sec. Preparations irradiated for 90 sec. showed no detectable infectivity and were employed for the present experiments.

Isotope incorporation. Primary or secondary monolayer cultures of chick embryo cells were prepared in 6 cm. Petri dishes containing a 24×40 mm. coverslip. After 2 days the exponentially growing cells were inoculated with infective VSV or u.v.-VSV. After adsorption for 50 min. at 37° the unadsorbed inoculum was removed and replaced by 2 ml. of warm culture medium. At various times the cells were pulse-labelled for 10 or 20 min. by addition of 0.5 ml. of the medium containing 0.5 to 2 #c of each radioactive compound. The cells on coverslips were then washed with cold 5 % trichloracetic acid and the residual radioactivities of the cultures were determined in a Beckman liquid scintillation counter; results are expressed as the mean value from two cultures. In experiments with u.v.-VSV the input multiplicities were derived from the virus infectivity before the u.v. irradiation.

RESULTS

Patterns of macromolecular syntheses in the cells infected with active or u.v.-VSV

The patterns of macromolecular syntheses in the cells seeded with infective VSV or u.v.-VSV are shown in Fig. 1 and 2, respectively. At zero time, coverslip cultures of cells were infected at a multiplicity of 500 p.f.u./cell. After adsorption for 50 min. at 37° the unadsorbed inoculum was removed and replaced by 2 ml. of warm culture medium. At different times the cells were pulse-labelled with 0.5 #c of $[14C]$uridine, 1 #c of $[14C]$thymidine or 0.5 #c of $[14C]$amino acids for 20 min. Control cells were similarly treated without VSV and the rates of incorporation represented as percentages of that for control cells. Cellular RNA synthesis was inhibited rapidly (Fig. 1) after infection with VSV, followed by an increase at 6 to 10 hr representing virus RNA synthesis, as described below. The inhibition of DNA synthesis started at about 2 hr after infection, (for detail see Fig. 7). In contrast, reduction of the rate of protein synthesis was reduced gradually. In the one step growth of VSV in chick embryo cells the latent period was about 1½ hr, and virus production reached a maximum at about 10 hr.

The same inhibitory effects on the syntheses of cellular macromolecules were shown by u.v.-VSV (Fig. 2) but not by VSV heated at 56° for 20 min.

The patterns of inhibition of cellular RNA synthesis by infective VSV and u.v.-VSV are compared in Fig. 3. Chick embryo cells were infected with active or u.v.-VSV at each input multiplicity of 300 p.f.u./cell. For determination of the rate of virus RNA synthesis, another set of coverslip cultures was infected with infective VSV or u.v.-VSV (100 p.f.u./cell) in the presence of 2 #g/ml. of actinomycin D. The incorporation of $[14C]$uridine was determined as in the experiment shown in Fig. 1. It is apparent that the peak of RNA synthesis at 6 to 10 hr after infection with infective VSV represents virus RNA synthesis. In contrast, no detectable RNA synthesis was induced by u.v.-VSV in the presence of actinomycin D.
DNA and RNA shut-off by u.v.-VSV

(Fig. 3). These results also indicate that both infective VSV and u.v.-VSV exhibited an equivalent shut-off activity on cellular RNA synthesis under the present experimental conditions.

Fig. 1. Inhibition of macromolecular syntheses in chick embryo cells infected with vesicular stomatitis virus. Each point is shown at the mid-time of the labelling period. △—△, [14C] amino acids; ●—●, [14C] thymidine; ○—○, [14C] uridine.

Fig. 2. Inhibition of macromolecular syntheses in chick embryo cells seeded with u.v.-vesicular stomatitis virus (irradiated for 90 sec., 500 p.f.u. u.v.-VSV/cell). Experimental conditions as for for Fig. 1. △—△, [14C] amino acids; ●—●, [14C] thymidine; ○—○, [14C] uridine.

U.v. dose and shut-off activities

The effects of different doses of u.v. irradiation on shut-off activities and infectivity of VSV are shown in Fig. 4. VSV suspensions in PBS (0.5 ml., 7 × 10⁶ p.f.u./ml.) were irradiated in shaking 6 cm. Petri dishes by the use of a germicidal lamp of 15 w at 90 v at a distance of 30 cm. Chick embryo cells were seeded with the u.v.-irradiated VSV preparation at an input multiplicity of 500 p.f.u./cell. RNA shut-off activity was determined by pulse-labelling with 0.5 μc/dish of [14C] uridine for 20 min. at 2 hr after infection. DNA shut-off activity was determined by pulse-labelling with 1 μc/dish of [14C] thymidine for 20 min. at 6 hr after infection. Virus activities were determined and the surviving fractions plotted as a function of u.v. dose. The infectivity of VSV decreased during irradiation at an average rate of
50%/sec. (Fig. 4), whereas the shut-off activities were quite resistant to irradiation. It was noteworthy that VSV retained the equivalent shut-off activities, even after irradiation for 3 min., when the infectivity had been destroyed completely. This makes it improbable that the expression of the virus genome in the infected cell is necessary for shut-off.

**Input multiplicity required for shut-off**

The relationship between the input multiplicity of u.v.-VSV and the shut-off activities on cellular DNA and RNA synthesis is shown in Fig. 5. Chick embryo cells were infected with twofold serial dilutions of u.v.-VSV (irradiated for 90 sec.). The rates of DNA and RNA synthesis were determined by pulse-labelling with [14C]thymidine or [14C]uridine for 20 min. at 6 or 4 hr after infection, respectively. A multiplicity of about 250 p.f.u. u.v.-VSV/cell was required (Fig. 5) for an apparent 50% reduction of RNA synthesis at 4 hr after infection. At that multiplicity, the rate of DNA synthesis at 6 hr after infection was about 30% of that in control cells.
Early kinetics of RNA shut-off

Chick embryo cells were treated with u.v.-VSV (irradiated for 90 sec. 500 p.f.u./cell) at 4° for 30 min. After removal of the unadsorbed inoculum, 2 ml. of culture medium at 37° was added to one set of cultures, and the cells were quickly transferred to an incubator at 37°. The unadsorbed inoculum in another set of cultures was replaced by cold medium and held at 4° for 1 hr before the cells were transferred to 37°. Control cells were similarly treated without VSV. At different times after the temperature shift, the cells were pulse-labelled with 0.5 μC/dish of [14C]uridine for 10 min., and the results represented as a percentage of incorporation into control cells. The patterns thus obtained (Fig. 6) indicated clearly that the RNA shut-off did not proceed at 4°, and started within 30 min. of transfer to 37°.

Fig. 4. Effect of different doses of u.v. irradiation on the infectivity and shut-off activities of vesicular stomatitis virus particles. •--•, DNA at 6 hr; ○--○, RNA at 2 hr; ▲--▲, surviving fraction of VSV.

Fig. 5. Relationship between shut-off activities on synthesis of cellular nucleic acid and input multiplicity of u.v.-vesicular stomatitis virus. ○--○, RNA at 4 hr; •--•, DNA at 6 hr.
Nature of DNA shut-off

Cover-slip cultures were treated with fresh culture medium or with u.v.-VSV (500 p.f.u./cell) at zero time. The cells were pulse-labelled with 2 μCi/dish of [14C]thymidine for 20 min. at different times. Evidently, with control cells mock-infected with fresh culture medium alone, stimulation of cellular DNA synthesis occurred after 2 hr, whereas no such stimulation was observed in u.v.-VSV seeded cells (Fig. 7). Thus, the apparent shut-off of cellular DNA synthesis (Fig. 1, 2) was due mainly to the inhibition of the stimulation of cellular DNA synthesis that was found in the mock-infected control cells.

Stability of cellular nucleic acids during u.v.-VSV infection

Coverslip cultures of cells were labelled with 0.01 μCi/dish of [14C]thymidine for 22 hr or 0.05 μCi/dish of [14C]uridine for 30 min. After labelling, the cells were repeatedly washed with PBS, then seeded with u.v.-VSV (irradiated for 90 sec., 500 p.f.u./cell) containing 100 μg/ml. of cold thymidine or uridine, respectively. The radioactivities of material insoluble in 5% trichloracetic acid were determined at different times (Fig. 8). There was no trend to the degradation of pre-existent cellular DNA or RNA to the acid-soluble state in the infected cells, although some residual incorporation of [14C]uridine was observed in the control cells.
DISCUSSION

When monolayer cultures of chick embryo cells were seeded with infective vesicular stomatitis virus or u.v.-VSV, cellular RNA synthesis was inhibited rapidly after the initiation of virus penetration into the cells. Reduction of the rate of protein synthesis emerged gradually and was less than that of RNA synthesis. The stimulation of cellular DNA synthesis, which was observed in control cells after medium replacement, was also inhibited by u.v.-VSV. This stimulation of DNA synthesis seemed to be due to the partially synchronized onset of S phase induced by medium replacement (Yaoi & Amano, unpublished observations; Gurney, 1969). When the cells were infected with u.v.-VSV at the time of medium replacement, subsequent onset of S phase was effectively blocked, whereas the level of already established DNA synthesis was not reduced significantly (Fig. 7).

![Graph](image)

Fig. 8. Stability of cellular nucleic acids during treatment with u.v.-vesicular stomatitis virus. (a) DNA; (b) RNA. ●●, control; ○○, infected.

The present results are in accordance with previous reports that cellular RNA synthesis is rapidly inhibited by infection with u.v.-VSV (Huang & Wagner, 1965; Wagner & Huang, 1966). One difference, however, is that a much higher multiplicity of u.v.-VSV is required to obtain sufficient shut-off than that required with the INDIANA serotype of VSV and suspensions of Krebs-2 cells (Wagner & Huang, 1966). It is not yet clear whether this discrepancy is due to differences in the purity or serotype of VSV preparations, or in the cell type or culture condition used in these experiments. U.v. irradiation had little effect on the capacity of VSV to adsorb to the cells at the dose employed in this experiment (90 sec.). The present VSV preparation contained few defective T-particles (Huang et al., 1966), as examined by sucrose density centrifugation and electron microscopy.

Despite infection at high input multiplicities, cytopathic effect, as reported by Cantell
et al. (1962), was not apparent until about 15 hr after infection with u.v.-VSV, and no de-
geradation of pre-existent cellular nucleic acids to the acid-soluble state was observed in the
infected cells for at least 6 hr (Fig. 8). Moreover, a peak of virus RNA synthesis was
observed, even when the cells were infected with as much as 500 p.f.u/cell of infective VSV
(Fig. 1). Thus metabolic capacities in the infected cells, at least to sustain virus multiplication
remained unchanged during the experimental period. This evidence suggested that the
shut-off effect was primarily restricted to host RNA synthesis and was not due to non-
specific damage to the cells caused by infection at high multiplicity.

As described already, the shut-off activities of VSV were quite resistant to u.v. irradiation
and were virtually unaffected even after the replicating activity of virus RNA had been
destroyed completely by irradiation (Fig. 3: u.v.-VSV + actinomycin D). It may be concluded
that some u.v.-resistant components in infecting virus particles are responsible for the present
shut-off phenomena.

We thank Dr K. Hara, Institute for Virus Research, Kyoto University, Kyoto, for valuable
discussions. The technical assistance of Mr T. Kawaguchi is also acknowledged.

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(Received 19 February 1970)