A Host-cell DNA Function Involved in the Replication of Avian Tumour Viruses and of Fowl-plague Virus

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(Accepted 2 December 1968)

SUMMARY

The multiplication of fowl-plague virus was much less sensitive to actinomycin D in chick cells infected with avian myeloblastosis virus and in hamster cells, transformed by Rous sarcoma virus than in comparable normal cultures. In addition, reproduction of fowl-plague virus was less effectively suppressed by u.v.-irradiation of cells carrying avian myeloblastosis or Rous sarcoma virus than of control cultures. These findings could indicate that avian myeloblastosis and Rous sarcoma viruses induce a function of host-cell DNA, whose products can be utilized in reproduction of fowl-plague virus.

INTRODUCTION

The reproduction of most RNA viruses, including Newcastle disease virus, is known to be resistant to actinomycin D (Barry, 1964). Nevertheless, some of RNA-containing viruses are inhibited by actinomycin D: viz. Rous sarcoma virus (Temin, 1963, 1964a; Bader, 1964, 1965), influenza viruses, including fowl-plague virus (Barry, Ives & Cruickshank, 1962; Barry, 1964) and murine leukaemia viruses (Bases & King, 1967; Duesberg & Robinson, 1967). Double-stranded RNA of poliovirus is more sensitive than single-stranded RNA (Koch, Quintrell & Bishop, 1967).

Sensitivity or resistance of RNA-viruses to actinomycin D is also reflected by u.v.-sensitivity of the host cells to grow the virus. The capacity of chick cells to support multiplication of Rous sarcoma virus was found to be extremely sensitive to u.v. light (Rubin & Temin, 1959) as was the capacity to reproduce influenza viruses (Barry, 1964). The capacity of chick-embryo cells to support multiplication of Newcastle disease virus is highly resistant to u.v. light (Rubin & Temin, 1959; Rosenberg & Rosenbergová, 1962).

The simplest explanation of these findings is that a host-cell coded function, necessary for virus reproduction, is induced with Rous sarcoma, influenza and murine leukaemia viruses. If this host cell function is common to all three viruses, preinfection of a cell with one type should ensure that the subsequent multiplication of either of remaining two in that cell is more resistant to the effects of actinomycin, because the essential host cell function is already present. Also the inactivation of cellular genes by u.v. light should prevent virus reproduction in a cell where the genes are repressed and their products absent but not in a cell where the products are already induced by another virus.

The following systems were chosen for study: RIF-free chick embryo cells and cells from the same batch infected with avian myeloblastosis virus. The virus multiplies and matures in the cells, but it does not transform them effectively. The baby hamster

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kidney cell line BHK/21, C13 (BHK) (Macpherson & Stoker, 1962), the same cells transformed by Rous sarcoma virus, strain SCHMIDT-RUPPIN, clone SR7 (Macpherson, 1965), and a strain derived from Rous sarcoma virus-transformed hamster embryo culture (RSH) were used (Veselý & Svoboda, 1965). Both of these cell lines are Rous sarcoma virus-transformed, but the virus does not mature in them. In these systems the presence of avian myeloblastosis or Rous sarcoma virus, does not interfere with fowl-plague virus. Fowl-plague virus was chosen as a representative of the influenza group, because it multiplies well in cell cultures of several different species and plaques may be produced in these cells.

**METHODS**

_Viruses._ Fowl plague virus, strain DOBSON, was obtained from the collection of this Institute. It was adapted to BHK cells by 10 passages; afterwards it was cloned by plaques in BHK cells and then regrown in allantoic cavity of chick embryo. Stock infectious allantoic fluid was dispensed in sister ampoules and kept at $-25^\circ$. A single virus batch was used in all experiments; no ampoule was thawed more than once. This virus stock contained $3 \times 10^8$ p.f.u./ml.; no decrease of infectivity was detected during 6 months of storage. Haemagglutination titre of this batch was 1:1024. Avian myeloblastosis virus, strain BAI-A, was kindly provided by Dr J. Řiman, Prague. Virus was propagated by intravenous inoculation of 1-day-old chickens. After the development of myeloblastosis the animals were bled into citrate solution. Infectious plasma served as virus stock; before infection of cell cultures the plasma was dialyzed against phosphate-buffered saline.

_Cells._ Chick embryo cells were prepared by trypsinization of 10-day-old embryos. RIF-free eggs were a generous gift of Dr I. Hložánek, Prague. One batch of cells was split in two parts. One of them was infected as primary culture with avian myeloblastosis virus (0.1 ml. plasma per $10^7$ cells). Both control and avian myeloblastosis virus-infected chick cells were passaged at the same intervals; for experiments the 3rd and 4th passage in vitro were employed. BHK and SR7 cells were kindly provided by Dr I. A. Macpherson, Glasgow. RSH cell line was obtained from Dr D. Šimkovič, Bratislava. All these cells were grown in ETC medium (Macpherson & Stoker, 1962), consisting of 8 vol. of Eagle's medium (Difco), 1 vol. of heated calf serum and 1 vol. of Tryptose-phosphate broth (Difco). Experiments with actinomycin D and with u.v. light were performed on confluent cell cultures, 3 days old, in 5 cm. Petri dishes. Chick embryo cells, both normal and preinfected with avian myeloblastosis virus, were seeded in 5 ml. of medium containing $10^8$ cells/dish. All three lines of hamster cells were seeded in 5 ml., containing $7 \times 10^6$ cells/dish. Confluent cultures contained (in terms of $10^8$ cells/5 cm. dish): chick cells, either normal or infected, 3.0 to 3.4, BHK 4.8 to 5.3, SR7 5.7 to 6.1, RSH 5.2 to 6.0.

_Plaque assay_ of fowl-plague virus was carried out in confluent chick embryo primary cell cultures, 2 days old, in 5 cm. Petri dishes. Each dish was seeded with $5 \times 10^6$ cells in 5 ml. of medium. Virus dilutions were prepared in phosphate-buffered saline, pH 7.2, containing 1% of heated calf serum. Virus inoculum 0.5 ml. per dish was adsorbed to the cultures for 1 hr at room temperature, with occasional shaking. After addition of agar overlay, the cultures were incubated for three days at 37°, stained with neutral red 1:10,000 in agar and the plaques subsequently scored.
**Cell DNA function common to RSV, AMV and FPV**

_U.v.-experiments_ employed a Philips TUV-30 w germicidal tube; the dose was monitored using a u.v. dose-meter (for 2537 Å), manufactured in this Institute by Dr E. Mrena. To avoid the possibility of photoreactivation, experiments were performed under dim yellow light.

*Actinomycin D* was a gift of Merck, Sharp and Dohme, Rahway, N.J.

**RESULTS**

*Actinomycin D*

Confluent cultures of respective cells, grown in 5 cm. Petri dishes, were used. After removing the growth medium, fowl-plague virus at a multiplicity of infection of approximately 0·1 p.f.u./cell was adsorbed to the cultures for 30 min. at room temperature. Unattached virus was removed, and the cultures were rinsed twice with phosphate-buffered saline containing 1% calf serum. ETC medium containing concentrations of actinomycin D ranging from 0 to 1·0 μg./ml. was added (5 ml./culture) and the cultures were incubated at 37° for 40 hr. At this time, the virus content of the medium was determined by plaque assay. Fowl plague virus production was less inhibited by actinomycin D in cells preinfected with avian myeloblastosis or with Rous sarcoma virus than in the respective controls (Fig. 1). Under these experimental conditions, fowl-plague virus yield (in terms of p.f.u./cell) in cultures without actinomycin D was similar in all types of cells used: chick cells RIF-free, 38; chick cells infected with avian myeloblastosis virus, 32; BHK, 21; SR7, 27; RSH, 8.
Inactivation of capacity by u.v. light

Experiments with u.v. light were carried out in a similar way. Confluent cultures of cells were grown in Petri dishes; after removing the medium the cultures were rinsed twice with phosphate-buffered saline to remove residual growth medium, which might shield the cells from u.v. rays, and the cultures were exposed to various u.v. doses. After irradiation, the cultures were infected with fowl-plague virus at m.o.i. approximately 0.1 p.f.u./cell. After 30 min. of adsorption, the inoculum was removed and the cultures rinsed twice, and 5 ml. of medium was added to each culture. After 40 hr incubation at 37°, the medium was harvested and the yield of fowl-plague virus was determined. The capacity to support the multiplication of fowl plague virus was more u.v.-resistant in cells preinfected with avian myeloblastosis or Rous sarcoma virus than in comparable normal cultures (Fig. 2). Also in this experiment the yield of fowl plague virus was comparable in all cultures, ranging from 14 to 30 p./cell.f.u.

Similar experiments were done with actinomycin D and with u.v. light using m.o.i. c. 5 and 0.01 p.f.u./cell. In experiments using 5 p.f.u./cell virus yield was determined after 20 hr incubation. All these experiments yielded results very similar to those presented above. At this multiplicity, however, virus yield in cultures untreated with either actinomycin D or u.v. light was in most instances less than 1 p.f.u./cell and it differed considerably between different types of cells; it was lowest in BHK.
DISCUSSION

The results presented here are consistent with expectation following from the model suggested in the Introduction. Nevertheless, there is no conclusive evidence that the model is correct. It will be necessary to show which gene products coded by the host cell are involved in the replication of both avian myeloblastosis or Rous sarcoma and fowl-plague viruses. There is known an example of host-cell coded and virus-induced enzyme, the uncoating enzyme of poxviruses (Joklik, 1964).

Avian leucosis viruses and myxoviruses have certain similarities, but these may be superficial. They include similarities in the multiplication cycle, abortive infections, defectiveness and to a certain extent a similarity in the structure of the virions. It has recently been reported that fowl plague virus grown in mixed infection with avian myeloblastosis virus showed double neutralization with antisera against both fowl-plague and avian myeloblastosis viruses (Závada & Rosenbergová, 1968), suggesting that phenotypic mixing had taken place. Nevertheless, there are some definite differences; whereas the reproduction of Rous sarcoma virus is sensitive to 5-iododeoxyuridine and cytosine-arabinoside, fowl-plague virus is resistant. This probably indicates that Rous sarcoma virus reproduction requires both functioning and replicating host-cell DNA, whereas fowl-plague virus requires only DNA functioning (Bader, 1965).

Some alternative explanations have been proposed for the facts found in the case of Rous sarcoma virus. To explain the unusually high u.v.-resistance of the infectivity of Rous sarcoma virus and the high u.v.-sensitivity of cell capacity to support its growth it was suggested that there is a genetic homology between the virus and the host cell (Rubin & Temin, 1959). Whether this explanation is true or not, in vitro hybridization of RNA extracted from avian myeloblastosis virus with DNA from uninfected chick cells apparently shows a relationship (Harel et al. 1966; Wilson & Bauer, 1967). The exact meaning of this finding for the viral replication cycle remains to be defined.

It has also been suggested that the RNA of Rous sarcoma virus after entering the cell serves as a template for a DNA copy which behaves as a provirus and in turn makes RNA strands (Temin, 1963, 1964a, b, 1967). This hypothesis has so far not been supported by convincing experimental data.

A third explanation is that it is not an essential enzyme that is coded by cellular DNA, but some type of RNA specified by host-cell DNA (Montagnier, 1968). This possibility should be considered seriously in devising further experiments.

Whatever the mechanism of the findings reported here, it is clear that they represent a new type of interaction between two viruses, analogous to complementation.

The author is grateful to Dr I. A. Macpherson, Glasgow, and Dr J. Huppert, Paris, for their critical reading of the manuscript, and to Mrs Eva Vrbová for expert technical assistance.

REFERENCES


(Received 18 September 1968)