KINETICS OF POLIOVIRUS REPLICATION IN HELA CELLS INFECTED BY ISOLATED RNA

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SUMMARY

Under conditions with the least toxicity for cells compatible with an optimal sensitizing effect for RNA infection, 47% of HeLa cells can be infected by viral RNA. Both RNA and virus infective centers produce identical amounts, i.e. 2000 PFU of progeny virus per infective center and both incorporate ^{3}H uridine in equal quantities. After infection with an effective multiplicity of ten PFU of virus or RNA, virus maturation occurs thirty minutes earlier in RNA-infected cells as compared to virus-infected cells.

INTRODUCTION

Exposure of HeLa cells to DEAE-dextran and DMSO (D+D) is one of the most effective methods to sensitize cells for infection by viral RNA (1). Incubation of cells at 37° C under these conditions has a toxic effect on the cells as shown by staining with trypan blue (1). Further experiments revealed that the capacity of cells to support a virus growth cycle is also impaired by D+D exposure (Oppermann and Koch, unpublished). Here we report on improved conditions for optimum sensitization of HeLa cells for RNA infection with the least toxicity for cells. Using these techniques, it was possible to compare events in cells infected with virus and RNA.

MATERIALS AND METHODS

Methods used for growing HeLa cells for the preparation of poliovirus Type 1, strain Mahoney, and viral RNA were described (2). Infection of cells by poliovirus was performed as described by Darnell and Sawyer (3). For infection with isolated viral RNA, cells were suspended in Eagle's minimum essential medium (MEM), buffered with

25 mM HEPES (N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid) (H-MEM) and sensitized by exposure to 10% DMSO and 400 µg DEAE-dextran/ml at a cell density of 10^7/ml at 37° C. A proper polycation/cell ratio is essential for optimal sensitization (5). After one minute exposure, poliovirus RNA was added and the mixture incubated for five more minutes. Then the cells were centrifuged and resuspended at 1-2 x 10^6/ml in H-MEM with 5% fetal calf serum (FCS). Actinomycin (5 µg/ml) and ^{3}H uridine (5 µC/ml and 2 µg uridine/ml) were added and during the following incubation, incorporation of ^{3}H uridine into TCA-precipitable material was measured according to a method described by Trown and Bilello (4). Infective centers induced by virus-infection or by RNA infection were determined by plating as described (3, 5). Microscopic examination of the cells revealed no signs of agglutination. To follow virus maturation, samples of the cell culture were lysed by 1/10 or 1/100 dilution into 0.5% NP_{40} (Nonidet P_{40}, Shell) in water and after further appropriate dilutions assayed as described by Bishop and Koch (6).

RESULTS

Cellular competence for viral RNA infection is not stable at 37° C (1) and only the competent cells in a given cell population can be infected by viral RNA at any one time. One infectious RNA molecule is able to induce a virus growth cycle in one cell (5) and all competent cells can be infected by RNA provided that they interact with at least one infectious RNA molecule. In a previous study (7), we found maximally 25% of HeLa cells to be competent for infection by viral RNA after cell sensitization by D+D whereas all cells from a HeLa cell culture could be infected by intact virus. Variations in the conditions for sensitization of cells (higher cell density, lower dose of DEAE-dextran/cell, shorter exposure time of cells to RNA) resulted in an increase in the yield of competent cells (up to 47%) and in a concomitant decrease in the toxicity for cells. This allowed us to

compare the kinetics of RNA synthesis and virus maturation in RNA and virus-infected cells. Cells sensitized by D+D were infected with an effective multiplicity of 1 or 10 PFU of RNA or 10 PFU of virus. In a parallel control experiment, a sample of untreated cells was infected with a multiplicity of 10 PFU of virus. One hour after infection, the yield of infective centers was determined. In the control culture all cells registered as infectious centers. From the sensitized cell culture 80% were infected by intact virus, 47% and 35% were infected by 10 or 1 PFU of RNA respectively. A comparison of the two virus-infected cultures reveals no effect of D+D exposure on the kinetics of viral RNA synthesis (Fig. 1). However, in this and many other experiments, we observed a delay in virus maturation of 10 to 15 minutes in the virus-infected D+D-treated cells. Provided the multiplicity of infection (m.o.i.)in RNA and virus-infected cells is comparable, the kinetics of RNA synthesis in RNA and virus-infected cells resemble each other. In contrast, virus maturation in RNA infected cells(m.o.i. of 10)precedes the virus maturation in virus-infected cells(m.o.i. of 10)by 30 minutes. The 1/2 to 3/4 hour time difference in virus maturation and RNA synthesis in the two cultures infected with 1 PFU or 10 PFU of RNA/cell is comparable to the observed time interval in cell cultures infected with 10-fold different multiplicities of infection with intact virus (8, 9). Both RNA and virus-infected cells produce identical amounts, i.e. 2000 PFU of progeny virus (corresponding to approx. 2 pg of virus) per infective center under our experimental conditions (Fig. 1).

DISCUSSION

Previously published data (5, 7) indicated that the low cellular competence for RNA infection was not due to limited RNA adsorption and uptake of RNA by the cells, but mainly caused by an intracellular event which restricted the mRNA function of viral RNA. Further studies have

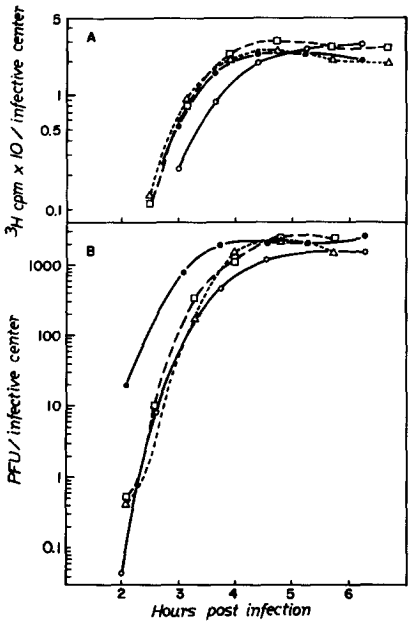

Fig. 1. RNA synthesis and virus growth in RNA- and virus-infected HeLa cells.

Samples of 10^7 cells were suspended in one ml H-MEM containing 400 µg DEAE-dextran/ml and 10% DMSO and incubated for one minute at 37° C and for five more minutes after addition of 2 µg (o---o) and 20 µg (•---•) isolated poliovirus RNA. Then the cells were sedimented and incubated in fresh H-MEM + 5% FCS at a concentration of 10^6/ml at 37° C. Another aliquot of 10^7 cells was exposed to D+D for six minutes parallel to the RNA-infected cell culture, the cells were sedimented and resuspended in fresh H-MEM at 10^7/ml and exposed to 20 PFU virus/cell for 30 minutes at 37° C, resulting in an effective multiplicity of about 10 PFU/cell (□---□). In parallel, we infected a control sample of 10^7 cells, not exposed to D+D, with virus under identical conditions (Δ---Δ) to determine the effect of D+D on virus replication. After 30 minutes exposure to the virus, both cultures were washed twice and incubated at 10^6 cells/ml. RNA syntnesis (Panel A) and virus maturation (Panel B) were determined in the four cultures as described in "Methods".

shown that all conditions used to increase the cellular competence for RNA infection interfere with cellular protein synthesis (10). DEAE-dextran in concentration of 40 µg/10^6 cells inhibits macromolecular synthesis of uninfected and infected HeLa cells reversibly (11). When cells are exposed to this amount of DEAE-dextran, 80% of the cells form virus-induced infectious centers, and 47% can be infected by viral RNA under these conditions. When a sample of the same cell population is

of glucose-6-P metabolism. The facts that these enzymes are induced by galactose as well as lactose, and that galactokinase and galactose-1-P uridyl transferase could not be detected, suggest that galactose is also metabolized via galactose-6-P, presumably by being phosphorylated at C-6 with phosphoenolpyruvate.

Our demonstration that galactose-6-P is metabolized through tagatose-6-P and tagatose-1,6-P_2 in *S. aureus* is contrary to the preliminary results of Simoni and Roseman (24), who suggested that the pathway in this organism involves conversion of galactose-6-P to 6-phosphogalactonate.

A detailed description of the purification and properties of galactose-6-P isomerase, tagatose-6-P kinase, and tagatose-1,6-P_2 aldolase will be presented elsewhere. The extent of the occurrence of this new pathway in other organisms is currently under investigation.

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attempts to introduce and translate other recently isolated mRNA's (interferon, hemoglobin, immunoglobulin) in intact cells.

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