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Early Events in the Lytic Infection of Primary Mouse Kidney Cell Culture with Polyoma Virus. The Effect of Various Input Multiplicities (39328)

ARNOLD BROWN
(Introduced by R. A. Consigli)
U.S. Veterans Administration Hospital, Pittsburgh, Pennsylvania 15240

Polyoma (Py) virus, a papovavirus containing circular double-stranded DNA, has been extensively studied in both the lytic and abortive infection, in this and other laboratories. Nevertheless, the precise sequence of events occurring in early infection and the precise interaction of the virus with its host, remains yet to be elucidated.

"Early" Py-RNA is defined as the species of viral-specific RNA which is found in lytically infected cells before the onset of viral DNA synthesis, or in cells in which DNA synthesis is blocked (5, 7, 10). In this report, we describe the effect of various input multiplicities on the synthesis of early viral RNA. The data suggest that: (i) the rate of RNA synthesis with respect to various input multiplicities is different early in infection (i.e., 30 hr at 27°) than it is later (i.e., 65 hr), and (ii) the interaction of more than one virus particle with each cell appears to be required for the earlier appearance of virus-specific RNA.

Materials and methods. The preparation of the virus stocks and the primary mouse kidney cell cultures (3, 10, 11), the modified procedure for RNA–DNA hybridization (1), and the technique for virus titration (6), have all previously been described. The RNA was extracted by the hot phenol method (8, 10) and four "cycles" of ethanol precipitation. The RNA–DNA hybridization was performed in 4 × SSC at 65° for 40–42 hr.

After viral adsorption, the cells were maintained at 27° (to slow the process of infection), in the presence of FdU (6×10^{-5} *M*) and in the absence of serum (to block DNA synthesis). The exact conditions and the characteristics of infection under these conditions have also been described previously (3, 5, 9). At the times indicated, the RNA was labeled with 250 μCi of [^{3}H]uridine (NEN, sp act 25 Ci/mmol) per ml of Dulbecco's reinforced Eagle's medium for a period of 3 hr, at which time the total cell RNA was extracted. The amount of RNA was estimated by measuring the absorbency at 260 μM in a Zeiss PM-2 spectrophotometer and the specific activity (cpm/μg RNA) was calculated. In most instances, 60 μg of RNA was used per hybridization, with DNA in excess.

Acid-soluble nucleotide pool determinations were performed essentially as described by Consigli and Ginsberg (4). Cells were infected at an m.o.i. of 20, or were "mock-infected," and were incubated at 27° for 65 hr in the presence of FdU. The medium was then replaced with fresh medium containing 0.5 μCi/ml of [^{14}C]uridine (NEN, sp act 50 mCi/mmol). After an additional 3-hr incubation, perchloric acid (PCA) extracts were prepared, concentrated by charcoal adsorption and elution, and hydrolyzed in formic acid at 175° for 45 min. After chromatography (isopropanol: HCl (conc):H_2O = 170:41:39), the spots were identified under a uv lamp, excised, and extracted with 0.1 *N* HCl. After uv spectrophotometry, aliquots were spotted on filter papers, dried, and the radioactivity was measured.

Results. Figure 1 shows the percentage of labeled RNA hybridizable to Py-DNA at times up to 65 hr after infection (27°, FdU) and input multiplicities ranging from 10^{-4} to 2×10^2 PFU per cell. At 27°, Py-RNA first becomes detectable at about 20 hr after infection. The amount of Py-RNA (percentage labeled RNA hybridizable to Py-DNA), synthesized at 65 hr after infection, approaches a linear relationship with respect to increasing m.o.i.s above 1. The amount of Py-RNA synthesized at lower m.o.i.s is consistent with that expected in the small proportion of cells which are infected. The relationship of the increasing m.o.i. to the

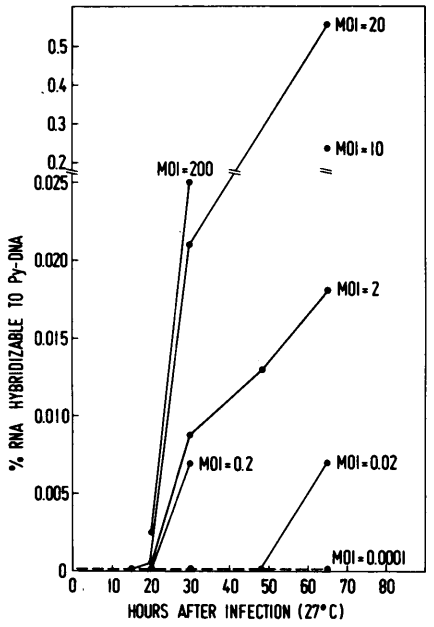

FIG. 1. The percentage of total RNA hybridizable to nitrocellulose filters containing an excess of Py-DNA is plotted for various multiplicities of infection. The infection was carried out at 27°, in the presence of 6×10^{-5} *M* FdU. At the times indicated, the cultures were "pulsed" for 3 hr (at 27°) with [^{3}H]uridine after which the RNA was extracted. The precise techniques have been described previously (see Materials and Methods). The radioactivity adhering to filters without DNA (blanks of 20–30 cpm) have been subtracted from the total counts per minute hybridized. One-tenth percent hybridizable RNA represents approx 2000 to 4000 cpm. Before plotting the data, the levels found in mock-infected cells (0.002–0.004%) have been subtracted from the values found on infection.

amount of Py-RNA synthesis at 30 hr is different than at 65 hr in that it appears that a maximum level is approached at the higher m.o.i.s studied.

"Early" in Py infection, there is no detectable change in the total amount of RNA present within the cells (unpublished data). There is a striking increase in the specific activity of RNA labeling with time after infection and with increasing m.o.i. (data not shown, see Fig. 2). The relationship between the increase in specific activity of RNA labeling and the logarithm of the amount of Py-RNA synthesized is shown in Fig. 2. To investigate the cause of this increase in RNA specific activity, the uracil pool size and rate of labeling was studied. Acid-soluble nucleotide pool determinations (3) were performed at 60 to 65 hr after infection (27°) following a 3-hr "pulse" with [^{14}C]uridine. The cells infected at an m.o.i. of 20, at 65 hr, contain 40% less PCA soluble uracil as compared with mock-infected cells. In addition, the infected cells incorporated 1.4 times as much labeled uridine from the medium. Both of these changes together lead to a sufficient increase in the specific activity of labeling of the uracil pool to account for the 2.1-fold increase in RNA labeling under the conditions cited (m.o.i. = 20 and T = 65 hr). A more detailed analysis of nucleotide pool changes will be forthcoming in a subsequent publication.

Discussion. At 27°, in the presence of FdU, Py virus infection at various input multiplicities, is associated with the synthesis of an amount of Py-specific RNA which is at m.o.i.s in which most or almost all the cells would be expected to be infected, more nearly quantitatively proportional to "gene-dose" at 65 hr than at 30 hr after infection. This may be due to the following: (i) the "saturation" of existing (viral) RNA-synthesizing capacity early with an increase of

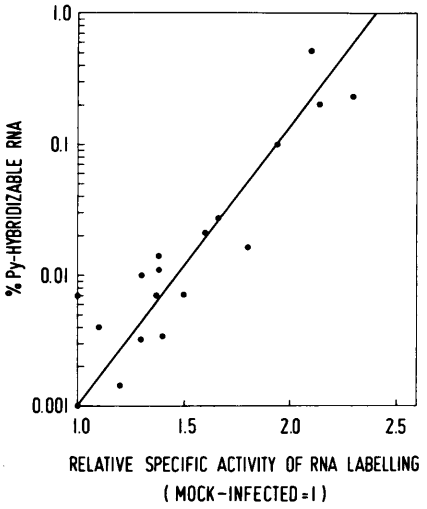

FIG. 2. The relationship between the amount of polyoma-specific RNA (see legend to Fig. 1) and the stimulation of relative specific activity of RNA labeling. (Cpm/μg RNA on infection − cpm/μg RNA of mock-infected cells.) Note the semilogarithmic coordinates which were used for convenience in plotting the data.

this capacity later; and (ii) it is possible that the rate of uncoating of the adsorbed virus DNA (therefore, its availability as template for transcription) is limiting. More complex explanations are also possible, but not probable. There appears to be a delay in the appearance of Py-RNA when the m.o.i. drops from 0.2 to 0.02. These values are associated with a drop in the numbers of total virus particles from approx one per cell to less than one per cell. The DNA contained in these nonplaque-forming virus particles may be less efficient templates for transcription. The hypothesis that the DNA in the defective particles may serve as a template for transcription is supported by preliminary experiments regarding the kinetics of T-antigen production (as determined by the proportion of nuclei stained using a fluorescent antibody technique), which we find is similar to that of early Py-RNA with respect to the m.o.i.

The changes in uracil pool size and the rate of uptake of uridine from the medium, are probably responsible for the increase in the specific activity of RNA labeling, which in turn is related to early Py genetic expression. It is conceivable that some change in the cell membrane affecting its permeability might be responsible for this effect.

Summary. Early polyoma (Py) virus-specific RNA synthesis was examined in cells infected with different concentrations of Py-virus. The effect of various multiplicities of infection (m.o.i.) on the rate of Py-RNA synthesis is different at 30 hr as compared to 65 hr. Thirty hours after infection at 27°, in the presence of 5-fluoro-2-deoxyuridine (FdU), an increase in input multiplicity was not associated with a quantitatively commensurate increase in the amount of virus-specific RNA synthesized. At 65 hr, the amount of viral RNA synthesized was roughly proportional to the number of infecting virus particles.

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