Incorporation of Iododeoxyuridine into Cellular Deoxyribonucleic Acid Synthesized After Infection with Simian Virus 40

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Primary monkey kidney cells (*Cercopithecus aethiops*) in the stationary phase of growth were labeled with ^{14}C -thymidine for 24 hr prior to infection with simian virus 40 (strain 777). ^3H -deoxyadenosine and 5-iodo-2'-deoxyuridine (IUdR) were added to some of the cultures 24, 48, or 72 hr after infection; 24 hr later the deoxyribonucleic acid (DNA) was extracted from these cultures and centrifuged in a CsCl density gradient. The portion of DNA which had become heavier because of incorporation of IUdR could be seen as a second peak in the sedimentation profile. This peak contained ^{14}C as well as ^3H activity. The possibility that the ^{14}C -labeled cellular DNA might be degraded and used for the synthesis of viral DNA could be excluded. On the basis of these results, it must be assumed that the infection of monkey kidney cells with simian virus 40 induces the synthesis of cellular DNA.

In earlier investigations, we were able to show that, after infection of stationary monkey kidney cell cultures (*Cercopithecus aethiops*) with simian virus 40 (SV40), besides viral deoxyribonucleic acid (DNA), a further DNA is formed which behaves like the DNA from noninfected cell cultures when chromatographed on a methylalbumin kieselguhr (MAK) column (8). This induction of cellular DNA synthesis was also shown to occur in cell cultures which were exposed to X rays prior to infection with SV40 (7). In contrast to the findings of Ben-Porat et al. (1) in corresponding investigations of mouse embryo cells after infection with polyoma virus, the DNA formed in the monkey kidney cells after infection with SV40 proved to be stable and was not degraded to smaller molecules during the subsequent course of infection.

This induction of cellular DNA synthesis after infection of the cell cultures with SV40 was also found by Hatanaka et al. (4), as well as by Kit et al. (5); however, Gershon et al. (3) reported different results. In view of these contradictory findings, we have searched for a further way to confirm our previous results experimentally. For this purpose, 5-iodo-2'-deoxyuridine (IUdR), together with ^3H -deoxyadenosine, was added to infected monkey kidney cell cultures in which the DNA had been labeled with ^{14}C prior to infection. If the cellular DNA which was labeled with ^{14}C prior to infection later incorporated

IUdR and if in addition this de novo synthesized DNA exhibited the properties of cellular DNA upon chromatography on an MAK column, it would have to be assumed that SV40 infection of stationary monkey kidney cell cultures leads to an induction of cellular DNA synthesis.

MATERIALS AND METHODS

Tissue culture. Primary monkey kidney tissue cultures (*C. aethiops*) were prepared according to the method of Youngner (10). The cell cultures used were grown either in Roux bottles (approximate cell count, 10^7 to 2×10^7) or, when stated, in petri dishes (approximate cell count, 10^6 to 2×10^6). Cultures were incubated in a humidified atmosphere and gassed with 5% CO_2 in air. A solution of 0.5% lactalbumin hydrolysate (Difco) in Hanks' balanced salt solution (BSS) was used as medium.

Virus. The virus used was SV 40 strain 777. The preparation of the virus suspension has been described elsewhere (8).

Infection and incorporation of IUdR. Stationary cell cultures were incubated with $0.08 \mu\text{C}$ of ^{14}C -thymidine (^{14}C -TdR) per ml (The Radiochemical Centre, Amersham, England; specific activity = 58 mc/mmole; $16.7 \mu\text{g}$ of ^{14}C -TdR per Roux bottle containing 50 ml of medium) for 24 hr before infection. After completion of this labeling process, the cell cultures were washed three times with Hanks' BSS and infected according to the method described by Werchau et al. (7); during this time, the same volume (50 ml) was added to the noninfected controls. The input multiplicity of infection was about 100 TCID_{50} per

cell. After an adsorption period of 2 hr, the virus which had not been adsorbed was removed, the cell cultures were washed again three times with Hanks' BSS, and new medium was added. Then $0.08 \mu\text{C}$ of ^3H -deoxyadenosine per ml (Schwarz BioResearch, Inc., Orangeburg, N.Y.; specific activity 3.8 c/mmole ; $0.316 \mu\text{g}$ of ^3H -deoxyadenosine per Roux bottle) and $10 \mu\text{g}$ of IUdR per ml (Farbwerke Hoechst) were added to different samples of the infected cultures and of the noninfected controls 24, 48, or 72 hr after infection. After an additional 24 hr, i.e., 48, 72, or 96 hr after infection of the cell cultures, the DNA was extracted from the respective cell culture samples and centrifuged in a density gradient of CsCl in a Spinco SW 39L rotor at $39,000 \text{ rev/min}$ for 24 hr. The extinction (optical density at $260 \text{ m}\mu$) and the ^{14}C and ^3H activities of the individual fractions were then determined.

DNA extraction. DNA extraction was performed according to Colter et al. (2); the percentage of recovery of DNA by the method used was 90%. After the extraction, the DNA was purified by the method of Marmur et al. (6).

Determination of radioactivity, chromatography on MAK column and titration of infectivity were carried out according to procedures described elsewhere (7, 8, 9).

RESULTS

In a first experiment, we determined whether an infected culture incorporates the same amount of ^3H -deoxyadenosine in the presence of $10 \mu\text{g}$ of IUdR/ml as cultures do without added IUdR. The results given in Fig. 1 show that the incorporation of deoxyadenosine was almost the same in both cases; furthermore, the production of virus during the time of the experiment was not affected by the addition of IUdR in this concentration.

Figure 2 shows the ^{14}C and the ^3H activities of the density gradients of the individual DNA samples from cell cultures to which ^3H -deoxyadenosine and IUdR were added 24, 48, or 72 hr after infection. The DNA from noninfected cultures to which no IUdR was added is represented by a single peak which contains ^{14}C as well as ^3H activities. The same result was obtained from investigations of DNA samples from infected cultures to which no IUdR had been added. However, in both the infected and the noninfected cultures to which IUdR was added, the incorporation of this thymidine analogue led to the formation of a heavier DNA which was represented by a second peak in the gradient centrifugation. This second peak contained a hybrid of IUdR-DNA and normal DNA. In some experiments, a small peak of a third kind of DNA was present, which may consist of double-stranded IUdR-DNA. Owing to the very small amount of this kind of DNA, this fraction was found only irregularly in repeated experiments.

The second peak containing a hybrid of

IUdR-DNA and normal DNA showed not only ^3H but also ^{14}C activities. Since the ^{14}C activity was incorporated solely into the cellular DNA before infection, it can be concluded from the above results that cellular DNA is also synthesized during infection.

When, under the same experimental conditions, $100 \mu\text{g}$ of IUdR/ml was added together with ^3H -deoxyadenosine and the DNA was subsequently examined by density gradient centrifugation, the same results were obtained (Fig. 3): the DNA which was heavier because of the incorporation of IUdR contained not only ^3H but also ^{14}C activity.

To determine whether the infection of stationary monkey kidney cell cultures with SV40 actually induces the production of cellular DNA, the ratio of ^{14}C counts in the two peaks of DNA

FIG. 1. Incorporation of ^3H -deoxyadenosine into the DNA of infected monkey kidney cells and into the DNA of noninfected controls; half of the cultures were given $10 \mu\text{g}$ of IUdR/ml simultaneously with the ^3H -deoxyadenosine (upper graph). Reproduction of SV40 (intra- and extracellular virus concentration) in monkey kidney cells which were given $10 \mu\text{g}$ of IUdR/ml after infection (lower graph). Symbols: $\times$ = infected cultures without IUdR (solid line) and with $10 \mu\text{g}$ of IUdR/ml (dashed line); $\bullet$ = noninfected cultures without IUdR (solid line) and with $10 \mu\text{g}$ of IUdR/ml (dashed line).

FIG. 2. Density gradient centrifugation of DNA samples from infected monkey kidney cells and from noninfected controls which were extracted at different times after infection. The culture cells had been labeled with ^{14}C -thymidine for 24 hr prior to infection, and after infection they were labeled with ^3H -deoxyadenosine at the given times. In addition, $10\text{ }\mu\text{g}$ of IUdR/ml was added to a portion of the cultures. Co. = noninfected control cultures; inf. = infected culture cells.

containing ^{14}C was measured. This measurement allows a calculation of the proportion of ^{14}C -DNA which has replicated. The results given in Table 1 demonstrate that the infection of the monkey kidney cell culture causes an induction of the synthesis of cellular DNA.

To confirm the assumption that the infection of stationary monkey kidney culture cells with SV40 induces the synthesis of cellular DNA, the possibility had to be excluded that cellular DNA which had been labeled with ^{14}C prior to infection of the tissue culture was degraded after the cells were infected and was then possibly used for the synthesis of viral DNA. For this reason, the DNA of the two peaks which were obtained in the experiment shown in Fig. 3 was denatured by heat and examined on an MAK column in the usual manner (Fig. 4). When the column was eluted with that concentration of sodium chloride with which native DNA as well as the greater part of the heat-denatured viral DNA, can be eluted (9), no ^{14}C activity could be detected in the effluent. From this result, it can be concluded that in in-

fected cultures the ^{14}C activity is incorporated exclusively into the cellular DNA.

DISCUSSION

The present report is concerned with the question of whether, in stationary monkey kidney culture cells infected with SV40, the synthesis of cellular DNA is induced along with that of viral DNA. Since there are conflicting reports in the literature on this subject, we have carried out further experiments. On the basis of our results, it must be concluded that the infection of the cultured cells leads to an induction of the synthesis of cellular DNA, since the DNA synthesized de novo after infection, which had in part become heavier due to incorporation of IUdR, contained ^{14}C activity along with the ^3H activity due the incorporation of ^3H -deoxyadenosine. The ^{14}C isotope, however, was given to the cells in the form of ^{14}C -thymidine solely before infection. The possibility that the cellular DNA labeled with ^{14}C prior to infection might have been degraded

FIG. 3. Density gradient centrifugation of DNA samples from infected monkey kidney culture cells and from noninfected controls. The cultures were labeled with ^{14}C -thymidine for 24 hr before infection and with ^3H -deoxyadenosine from 48 to 72 hr after infection; 100 μg of IUdR/ml was added simultaneously with the ^3H -deoxyadenosine.

FIG. 4. Chromatographic examination on an MAK column of the two DNA peaks obtained by density gradient centrifugation of extracts from infected monkey kidney cells (72 hr after infection). The culture cells were labeled with ¹⁴C-thymidine for 24 hr before infection and with ³H-deoxyadenosine from 48 to 72 hr after infection; 100 µg of IUdR/ml was added simultaneously with the ³H-deoxyadenosine. Only the ¹⁴C counts are shown in the figure.

and that the degradation products were then used for the synthesis of viral DNA could be excluded.

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TABLE 1. Proportion of replicated ¹⁴C-DNA of total DNA which can be extracted from infected and noninfected cultures at different times after infection^a

Culture	Percentage of ¹⁴ C radioactivity in hybrid IUdR-DNA after exposure to 100 µg of IUdR/ml	
	24-48 hr after infection	48-72 hr after infection
Noninfected...	4.0	1.3
Infected.....	25.6	9.3

^a Primary monkey kidney tissue cultures in petri dishes were labeled with ¹⁴C-thymidine (0.1 µc/ml; specific activity, 58 mc/mmole) from 72 to 24 hr before infection. After this labeling period, new medium was added, which was again used after infection as conditioned medium. At 24 to 48 hr and 48 to 72 hr after infection, 100 µg of IUdR/ml and ³H-deoxyadenosine (0.4 µc/ml; specific activity, 3.8 c/mmole) were added to the cultures. After this second labeling period, the DNA was extracted and banded in a density gradient of CsCl; the radioactivity of the different fractions was then determined. The proportion of ¹⁴C-labeled DNA that had become associated with the hybrid IUdR-DNA was evaluated.

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