*INDUCTION OF DNA SYNTHESIS BY SV40**

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The infection of cultures of confluent mouse kidney cells with the oncogenic polyoma virus causes the induction of cellular DNA synthesis and an increase in the activity of several enzymes involved in DNA synthesis.[1, 2] This induction is a direct action of the virus, since it only takes place in cells in which viral multiplication occurs.[3] Induction is absent or very limited in actively growing cultures,[4] for reasons that are not clear.

Simian virus 40 (SV40) is similar in size, structure, and DNA content to polyoma virus, and is also oncogenic. It differs from polyoma virus in the base ratios of its DNA,[5] and in the spectrum of host cells in which it can multiply or which it can transform. It is interesting to determine whether this virus also induces the DNA and enzyme syntheses of the host in order to establish the generality of the phenomenon. A recent report[6] suggests that SV40 lacks inducing activity. The results to be presented below, however, clearly show that SV40 has an inducing activity similar to that of polyoma virus, when it infects *resting* confluent cultures. Thus the two viruses behave very similarly in this respect.

The use of SV40 offers a special advantage over polyoma virus. Cells infected or transformed by SV40 contain in their nuclei the so-called T antigen, which is recognizable by either complement fixation or immune fluorescence with proper sera, and whose function is unknown.[7] A similar antigen is also present, but more difficult to demonstrate, in cells infected or transformed by polyoma virus. In cells in which SV40 multiplies lytically, the T antigen appears earlier than other recognizable viral products;[8] hence, it is interesting to determine the time relationship of the appearance of the T antigen and of induction, since it is possible that the synthesis of the T antigen may be the first viral function expressed in the cell, and that other consequences of infection may result from its activity. Our results will show that appearance of the T antigen precedes induction of cellular DNA synthesis.

Materials and Methods.—Virus and cell cultures: The strain of SV40 used is a small plaque variant, isolated by Dr. M. Vogt, from a strain kindly supplied by Dr. Sabin. The virus was propagated either in African green monkey kidney cell cultures or in cultures of the BSC-1 line derived from the same species. Virus purification was made according to a procedure devised by Dr. Axelrod.[9] When the cells became rounded, the medium was removed from the cultures, which were then washed with TD. The cells, collected by scraping with a rubber policeman and suspended in TD, were sonicated for 10 min and then treated with 0.01% trypsin and 1% sodium deoxycholate at 37° for 30 min; trypsinization was stopped by cooling to 4° and adding 20% serum. The suspension was spun down at 1,000 rpm for 10 min; the supernatant was placed above a layer of 8 ml of saturated potassium bromide in 0.01 *M* tris pH 7.5 and 0.01 *M* EDTA in the Spinco SW 25 rotor, which was spun for 3 hr at 25,000 rpm. Three bands were observed, the most dense of which contained the major part of the infectivity. The virus of this band was collected and frozen at −20° in Eagle's medium with 20% fetal bovine serum. BSC-1 cells and AGMK cells which were purchased from the Flow Laboratory were plated onto plastic Petri dishes in a reinforced Eagle's medium with 10% fetal bovine serum.

Infection of cultures: Infection was carried out as previously reported with polyoma virus, by using an inoculum containing 4×10^8 plaque-forming units per ml. The cultures were infected

when they had formed confluent layers, mostly of epithelial cells. The cultures were incubated for 1 hr at 37° in a well-humidified incubator flushed with a CO_2 air mixture; after this time, fresh medium was added. All control cultures were mock-infected under identical conditions but without virus.

DNA synthesis: DNA synthesis was determined from the amount of H^3-thymidine taken up either into acid-insoluble material[1] (in the presence of tenfold excess of uridine to minimize incorporation of the radioactivity in RNA) or in DNA extracted by SDS-phenol.[1] The infectivity of DNA was assayed by plaque formation[10] on confluent monolayer cultures of BSC-1 cells in 50-mm plastic Petri dishes. The cultures were first shocked with hypertonic saline (0.37 *M*, then 0.55 *M*) in 0.05 *M* tris pH 8, and then 0.2 ml of DNA in 0.55 *M* NaCl was added. After adsorption for 25 min at 29°, medium containing 20% fetal calf serum was added, and the plates were left at room temperature for 30 min. They were then transferred to the 37° humidified CO_2 incubator. After 19 hr the medium was sucked away, and the cultures were washed with 5 ml of Tris and overlaid with agar-containing medium. The plaque was counted after 22-day incubation.

Enzyme assays: Thymidine kinase, deoxycytidine, monophosphate deaminase, and DNA polymerase were assayed as described previously,[1, 12] except that time, temperature, and substrate concentration were slightly modified as described in the legends of the table and figures.

Immunofluorescence tests for the T antigen were carried out as described in the paper of Rapp *et al.*,[11] with antisera kindly supplied by Drs. Black and Girardi. Part of the serum was purchased from Flow Laboratory, Rockville, Maryland.

Results.—Rate of DNA synthesis after SV40 infection of AGMK cultures: The time course of DNA synthesis in infected and uninfected cultures was determined from the amount of thymidine incorporated in 12-hr periods at different times after infection. Figure 1 shows that the rate of thymidine incorporation into DNA began later than 24 hr after infection and declined at 48 hr postinfection. In uninfected cultures, in contrast, DNA synthesis occurred at a low rate, since the cultures were in stationary phase. The nature of the DNA synthesized, whether viral or cellular, was determined by using MAK column chromatography.

Figure 2 shows the result of one such chromatographic separation of viral and cellular DNA. DNA extracted from infected cells mixed with either SV40 or polyoma DNA as marker was heat-treated and chilled rapidly, then applied to the column. About 90 per cent of the radioactivity of the marker viral DNA and 80 per cent of the radioactivity of the DNA extracted from the cells were recovered by this technique. The peak eluting first contains the viral DNA and the following peak contains the heat-denatured cellular DNA. Viral DNA was also determined by its infectivity.

The relative rates of viral and cellular DNA synthesis at various times after infection are given in Table 1. The proportions of viral DNA, determined either by infectivity or chromatography, are consistent with each other. The data show clearly that induction of cellular DNA synthesis occurs following SV40 infection in the AGMK cell cultures during the lytic multiplication cycle and that synthesis of cellular DNA precedes synthesis of viral DNA.

Changes in enzyme activities: Figure 3 shows the change of activity of enzymes and DNA synthesis. The activities of thymidine kinase, dCMP deaminase, and DNA polymerase increase almost concurrently. It should be noted that the thymidine kinase activity, after reaching its maximum value, remains high, in contrast to polyoma-infected mouse kidney cells, in which it drops rapidly. The difference appears to be due to a difference of heat stability of the two enzymes.

Appearance of T antigen: Figure 4 shows the time course of the appearance of T

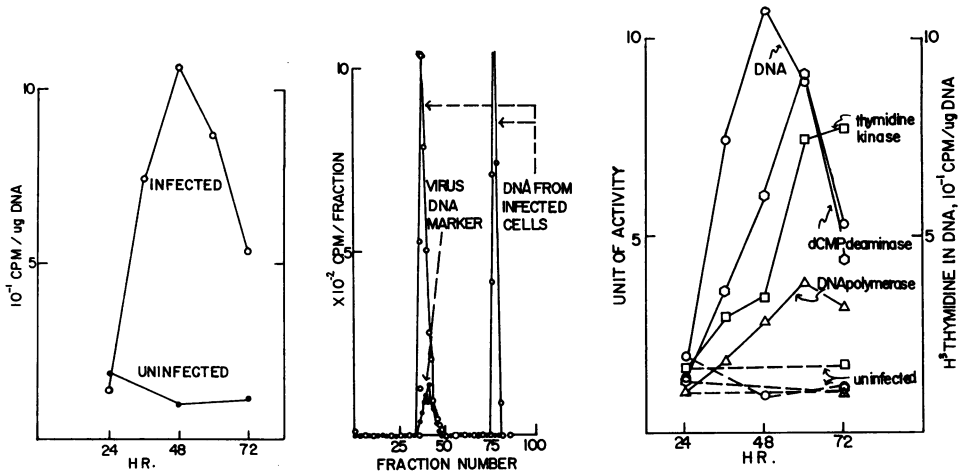

FIG. 1 (*Left*).—Time course of DNA synthesis in infected and uninfected cultures. Confluent cultures were infected as described in *Materials and Methods*. Twelve hours before the times indicated, 25 μc of H^3-thymidine (final concentration 5×10^{-5} *M*) was added to infected and uninfected cultures. Cultures were removed at various times, extracted with SDS and phenol, and the amount of radioactivity incorporated in DNA was determined. The measurement of total DNA and the determination of the specific activity were performed as described in the report of Munk *et al.*[6]

FIG. 2 (*Center*).—Fractions from a column of methylated albumin. The DNA of SV40-infected AGMK cells 72 hr after infection was extracted by SDS-phenol. The aqueous phase of the phenol extract (0.3 ml) was diluted to 10 ml with 10 times diluted SSC, and heated for 10 min in a boiling water bath, then chilled quickly in ice water. After adding 10 ml of 1 *M* NaCl buffered at pH 6.7 and P^{32}-labeled marker viral DNA, the sample was applied to the column. Each point represents the number of counts present in each fraction. The samples of fractions numbered 1–70 (2.5 ml/tube) were eluted by an NaCl gradient from 0.5 *M* to 0.8 *M* in 0.05 *M* phosphate buffer, pH 6.8. Then 300 ml of 2 *M* NaCl was added directly onto the column, and 25 ml of solution was collected in tubes 71–85. The radioactivity still remaining in the column (about 20% of the total input) was eluted by adding 2 *N* NH_4OH.

FIG. 3 (*Right*).—Changes of activities of enzymes and DNA synthesis. Activities of thymidine kinase (□—□), dCMP deaminase (○—○), and DNA polymerase (△—△) were assayed at various times. The scale of the ordinate is linear, in arbitrary units. Thymidine kinase was incubated at 37° for 5 min, dCMP deaminase at 37°for 20 min, and DNA polymerase at 37° for 40 min. H^3-thymidine incorporation into DNA (●—●) was described in Fig. 1. The broken lines show enzyme activities and DNA synthesis in uninfected cells. The reaction mixture of dCMP deaminase[12] was slightly modified as follows to obtain linearity vs. time and protein concentration: 0.05 ml of cell extract[12] and 0.25 ml of H^3-dCMP, 3×10^{-3} *M*, 10 μc/mg were mixed and assayed as described before.[12]

TABLE 1

DNA SYNTHESIS

Hours after infection	24	36	48	60	72
Cpm total DNA	2,715†	16,720	26,620	22,850	14,900
Cpm host DNA					
Infected	2,606	6,390	9,580	8,030	5,970
Control	1,835	—	1,950	—	2,560
Cpm viral DNA	109	10,330	16,840	14,820	8,930
(Mμmole H^3 TMP in viral DNA*)	0.007	0.702	1.143	1.008	0.616
Molecules viral DNA synth. $\times 10^{-11}$	0.02	1.45	2.36	2.08	1.27
Molecules of viral DNA integral‡	0.02	1.47	3.83	5.91	7.18
PFU of infectious DNA	0	76	132	320	470
PFU of infectious particles $\times 10^{-6}$	—	15	130	780	1,210

The experimental conditions for pulse-labeling by H^3-thymidine are the same as described in Fig. 1. The DNA extraction, heat treatment, and the separation of the viral DNA from cellular DNA by column chromatography were performed as described in Fig. 2.

* The radioactive H^3-thymidine used in this experiment had a specific activity of 1.47×10^4 cpm/mμmole. Assuming that thymidine is 29% of all DNA bases, 1 mμmole of TMP in DNA corresponds to 2.06×10^{11} molecules of viral DNA.

† Number of counts per culture.

‡ In order to compare the formation of infectious DNA to the amount of viral DNA separated on the column, the pulse-labeled viral DNA was integrated.

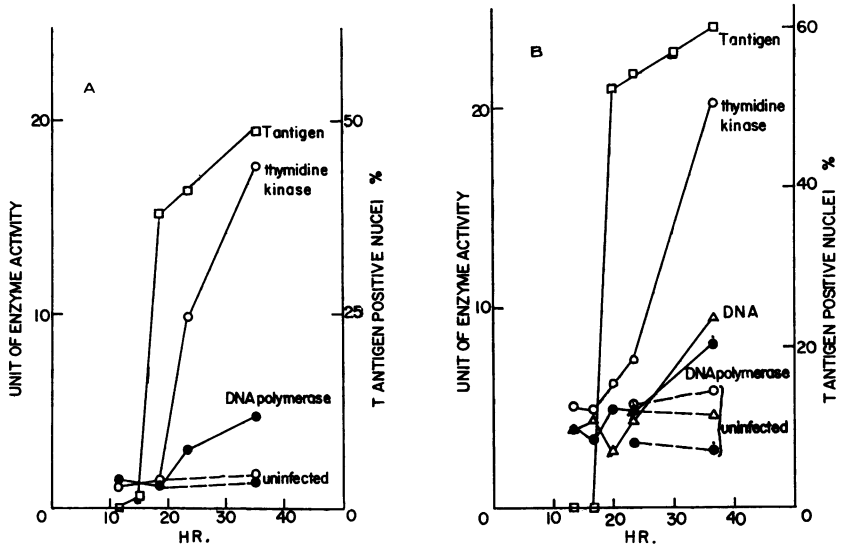

FIG. 4.—T antigen formation and changes of enzymatic activities. Immunofluorescence studies of T antigen were done according to the method of Rapp *et al.*[11] Cell cultures were grown on coverslips placed in Petri dishes. At specified times after infection, the coverslips were removed, rinsed, and fixed. The coverslip cultures were incubated at room temperature for 1 hr, then rinsed twice in PBS, once in distilled water, and mounted in PBS glycerine. The fluorescent nuclei were counted by scanning more than three optical fields. Enzymatic activities were assayed as described in Fig. 3, except that crude sonicated extracts were used for the enzyme assay in Fig. 4*B* instead of 10,000 × *g* supernate. In the case of Fig. 4*A*, 4×10^7 PFU per plate of the virus was used, while 2×10^8 PFU was used in Fig. 4*B*. One unit of relative activity corresponds to 1,000 cpm of radioactivity into the acid-insoluble fraction for DNA polymerase, 2,000 cpm of the phosphorylated thymidine compound formation for TK, and 5,000 cpm of the acid-insoluble fraction for DNA synthesis.

antigen determined by the proportion of nuclei with specific immunofluorescence, together with that of the induced enzymes. T antigen began to be formed 19–20 hr p.i.; the activities of thymidine kinase, dCMP deaminase, and DNA polymerase started to increase a few hours later. Thus the T antigen is the first demonstrable virus-controlled product to appear in the infected cells.

Summary.—SV40 induces cellular DNA and enzyme synthesis in the AGMK cells during a regular lytic cycle. The T antigen appears just before these syntheses begin.

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Abbreviations: SV40, Simian virus 40; AGMK, African green monkey kidney; TD, tris-saline buffer without divalent cation;[1] EDTA, ethylene diamine tetraacetic acid; MAK, methylated albumin kieselguhr; SSC, sodium citrate buffer,[1] 0.015 *M* with 0.15 *M* NaCl;[1] PBS, phosphate-buffered saline;[1] PFU, plaque-forming units; TK, deoxythymidine kinase; Tris, tris-buffered saline.[1]

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