SIMIAN VIRUS 40 RAPIDLY LOWERS cAMP LEVELS IN MOUSE CELLS.

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SUMMARY

The addition of SV40 to contact inhibited Balb/3T3 cells causes a 2-fold decrease in intracellular cAMP levels. The levels reach a minimum 3 hours after virus addition, and after a few hours begin to rise toward normal. No significant changes in cAMP levels are observed after cells are exposed to UV-inactivated virus or are mock-infected. This is the earliest known effect of SV40 infection. We propose that SV40 induces host DNA synthesis by lowering cAMP levels.

The papovavirus family consists of small DNA viruses that transform a variety of cell types. The addition of one of these, SV40, to arrested or contact inhibited cells induces cellular DNA synthesis and multiplication (1) Other agents which stimulate DNA synthesis in contact inhibited cells include serum, trypsin, and insulin. In these cases the stimulation of growth is always preceded by a fall in cyclic AMP levels (2,3). Here we report that induction of DNA synthesis by SV40 is also preceded by a transitory fall in cAMP levels. This decrease in cellular cAMP is the earliest known effect of SV40 infection on cellular metabolism. We believe the fall in cAMP is causally related to increased DNA synthesis.

MATERIALS AND METHODS

Balb/3T3 cells (cl A31) were grown in Falcon plastic tissue culture vessels in 10% CO_2, and fed twice weekly with Dulbecco-modified Eagle's medium + 10% calf serum (Colorado Serum Company).

Stocks of SV40 (small plaque) were made by inoculating confluent cultures of Vero cells with about 0.01 p.f.u./cell of plaque-purified SV40. The cultures were fed medium with 1% calf serum until the maximum cytopathic effect was obtained. The cells and medium were then frozen and thawed three times,

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and centrifuged for 10 min at 10,000 rpm in the Sorvall SS34 rotor. The supernatants were made 1% in sodium desoxycholate; layered over 10 ml of CsCl or KBr, 1.40 g/cm^3, in 0.01 M Tris, pH 7.4; and centrifuged for 3-4 hr at 22,000 RPM at 20^{o}C in the Spinco SW27 rotor. The viral bands were collected and dialyzed twice against medium.

SV40 was inactivated for 30 min with a GE 30 watt germicidal lamp; this reduced the pfu/ml about 300-fold. "Factor-free" medium (4) was prepared as described (5).

Cultures were analyzed by autoradiography (5): at least 600 nuclei were counted for each measurement. For the assays of cAMP the medium was aspirated from the plates and replaced with 1 ml of 5% CCl_3COOH (1^{o}C) containing ^{3}H-cAMP. The cAMP (6) and the total cellular nucleic acid (7) were measured as previously described.

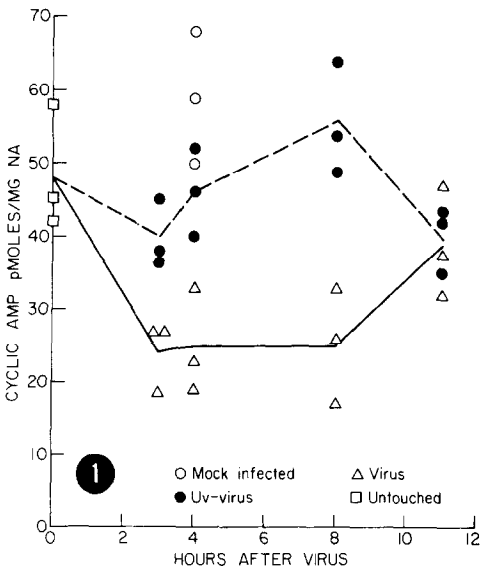
RESULTS

The stimulation of host DNA synthesis by SV40 is best observed in non-growing cells. We obtained resting cells by placing confluent cultures of Balb/3T3 cells in factor-free medium (4). The cultures were then infected with SV40. The cAMP content of the cells drops approximately two-fold within a few hours after infection (Figs. 1 and 2). No change in cAMP level is evident after 30 min; the level begins to fall within two hrs and reaches a minimum after three (Fig. 1). By 8-12 hrs the cAMP content has almost returned to normal. Serum lowers cAMP levels more rapidly than SV40 (Fig. 2).

Induction of cellular DNA synthesis by SV40 was monitored by autoradiography of cultures in parallel with those of Fig. 1. The SV40 preparation induced DNA synthesis in 16% of the cells. (Table I). The cAMP values are the average of cAMP in all the cells in the culture; there is no way to measure cAMP in individual cells. The induction of DNA synthesis and the fall in cAMP both require functional viral genomes; UV-irradiated virus is ineffective (Figs. 1 and 2, and Table I). (Growth factors in serum are not inactivated by this treatment). When a mock-infected culture is treated with 15% serum, 49% of the nuclei are labeled. The low induction by SV40 presumably reflects the low multiplicity of infection.

DISCUSSION

One of the cardinal facts about the biology of papovaviruses is the low information content of their genomes. The molecular weight of the



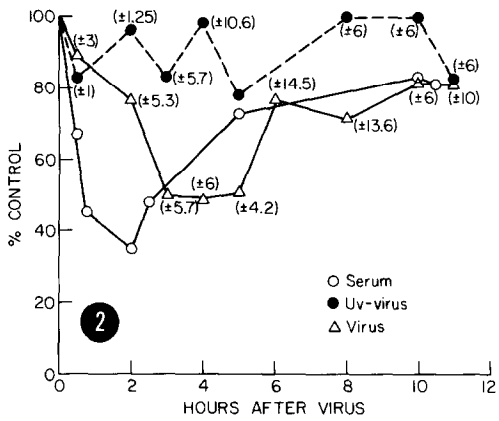


Figure 1. Effect of SV40 infection upon cAMP content of resting Balb/3T3 cells. Balb/3T3 cells were grown to confluence in 100mm tissue culture dishes (56 cm^2) and then incubated in 10 ml of factor-free medium for two days. This medium was then replaced with 3 ml of virus, UV-inactivated virus, or unsupplemented medium (mock infected). After two hours, these inocula were replaced with 10 ml of fresh factor-free medium. At the times indicated the plates, containing about 3 x 10^6 cells, were assayed for cAMP and total nucleic acid. The multiplicity of infection was approximately 2.5 p.f.u./cell. The time is recorded from the beginning of the 2-hour adsorption period. Each point represents one plate assayed in quadruplicate.

Figure 2. Effect of SV40 infection upon cAMP content of resting Balb/3T3 cells: compilation of 3 experiments. Cells were infected and harvested as described in the legend to Figure 1. Results for each plate (pMoles cAMP/mg nucleic acid) were normalized to mean levels found in untreated plates in the respective experiments. Each point is the mean of these percentages. Figures in parenthesis are standard error of the mean for each point.

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double-stranded DNA is about 3 x 10^6 (1). This DNA can probably code for no more than 10 proteins; the major virion protein alone accounts for approximately 25% of this genetic information (8). Thus, there can only be a small number of viral genes that control cell growth.

In this paper we show that SV40 lowers cAMP levels in Balb/3T3 cells, which can be transformed but will not support viral replication. This decrease in cAMP levels is the earliest known effect of papovavirus infection upon host cells. It occurs 2-3 hours after infection, whereas the synthesis

TABLE I

Induction of DNA Synthesis by SV40

Inoculum	% labeled nuclei
SV40	16
UV-inactivated SV40	3
Unsupplemented medium	2
Unsupplemented medium, then medium containing 15% serum	49

Cells were cultured in 35mm tissue culture dishes (8.5 cm^2). The cultures were seeded, grown and treated in parallel with those used in Fig. 1; each plate was initiated with 1/7 as many cells as the larger plates and was then treated with 1/7 the volume. After 2 hr, the inocula were removed and replaced with fresh factor free medium or medium supplemented with 15% serum (last row). The cultures were labeled with 2 μCi/ml of ^3H-thymidine from 12-48 hours after infection and were then fixed and processed for autoradiography as described.

of DNA and of enzymes related to DNA synthesis increase around 18 hours after infection (9; L. Rubinstein and A. Rein, unpublished). UV-irradiated virus does not produce the decrease, suggesting that the fall is due to expression of viral genes. The rapidity of the effect implies that it is closely connected to the primary effect of these genes.

The stimulation of cellular DNA synthesis by SV40 is also due to the expression of one or more viral genes, since (a) it is prevented if the virus is inactivated with ultraviolet light or nitrous acid (10,11,12); (b) it is caused by infection with purified viral DNA (13); (c) a temperature-sensitive mutant of polyoma virus, ts 3, induces cellular DNA synthesis at the permissive but not the non-permissive temperature (14).

Still another function of papovavirus genes is probably the maintenance of the transformed phenotype in permanently transformed cells. Studies with the ts 3 mutant (14,15) suggest that expression of the same gene is required for the early induction of DNA synthesis and the altered growth regulation seen in transformed cells.

The simplest hypothesis to explain our results is that the drop in cAMP level is causally related to the increase in cellular DNA synthesis. We further suggest that the same viral function which lowers cAMP levels and induces DNA synthesis soon after infection also acts to maintain low cAMP levels in cells permanently transformed by the virus. This would be in full accord with other evidence (2,3,6,7,16) that cAMP functions as an inhibitor or negative regulator of the growth of cultured fibroblasts.

SV40 infection caused a drop in levels 2-3 hours after infection, whereas serum, insulin, and trypsin act more rapidly (2). Presumably the delay represents the time required for the viral genome to enter the cell, be uncoated, and translated. Serum, insulin, and trypsin probably act directly upon the cell surface, which contains enzymes of cAMP metabolism.

Raska (17) recently reported that adenovirus 12 produced a dramatic fall in cAMP levels in G1-arrested BHK21 cells. He also found a decrease in adenylate cyclase acitvity. Interpretation of these experiments is complicated by the fact that the cells do not survive the infection. The effects of SV40 upon the enzymes of cAMP metabolism have not been studied.

The close connection between viral genes required for maintenance of the transformed phenotype and cAMP also occurs in chick embryo fibroblasts transformed by Bryan high titer Rous sarcoma virus. In such cells cAMP levels are low (18) and the activity of adenylate cyclase is decreased (19). Thus both DNA and RNA tumor viruses lower cAMP levels in their hosts. These results support the hypothesis that cAMP is involved in viral transformation.

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