On the functional roles of simian virus 40 large and small T-antigen in the induction of a mitotic host response

Gauchat, Jean-François; Weil, Roger

How to cite

GAUCHAT, Jean-François, WEIL, Roger. On the functional roles of simian virus 40 large and small T-antigen in the induction of a mitotic host response. In: Nucleic Acids Research, 1986, vol. 14, n° 23, p. 9339–9351. doi: 10.1093/nar/14.23.9339

This publication URL: https://archive-ouverte.unige.ch/unige:151574
Publication DOI: 10.1093/nar/14.23.9339

Volume 14 Number 23 1986 Nucleic Acids Research

On the functional roles of simian virus 40 large and small T-antigen in the induction of a mitotic host response

Jean-François Gauchat and Roger Weil

University of Geneva, Department of Molecular Biology, 30 Quai Ernest Ansermet, 1211 Geneva 4, Switzerland

Received 23 September 1986; Revised and Accepted 11 November 1986

ABSTRACT

The early gene of wild-type (wt) SV40 specifies two related proteins, referred to as large (Mr 88000) and small (Mr 19000) T-antigen. Infection with wt SV40 of Go/G1-arrested monkey kidney and CV-1 cell cultures induced in virtually 100% of the cells T-antigen synthesis, followed by a mitotic reaction and the production of SV40 DNA. Parallel cultures were infected with SV40 deletion mutants that produce either no small T-antigen (dl883) or only trace amounts of a truncated form (dl891). Kinetics of synthesis and accumulation of large T-antigen was closely similar to that observed with wtSV40 whereas apparently only 50-60% of the cells participated in the mitotic reaction and the production of viral DNA. These results and those obtained from a comparative study on the abortive (transforming) infection in Go-arrested mouse tissue culture cells indicate that synthesis of large T-antigen alone is sufficient to trigger in 50-60% of the infected cells a mitotic reaction.

INTRODUCTION

SV40 is a small oncogenic DNA virus wich induces tumors and leukemias in hamsters (1,2). Wild type (wt) SV40 contains as genome a double-stranded circular DNA of 5243 base pairs (bp) (3). The primary transcript of the early gene of wt SV40 undergoes two types of splicing: the removal of an intervening sequence (IVS), extending from 0.533-0.600 map units (346 bp) on the physical map of SV40 DNA, results in the mRNA coding large T-antigen (708 aa; Mr 88000), the major early viral protein; removal of an IVS extending from 0.533-0.546 map units (66 bp) leads to the mRNA which codes small T-antigen (174 aa; Mr 19000) (1,2,4). Infection with wt SV40 of quiescent tissue culture cells induces synthesis of SV40 large and small T-antigen, followed by a mitotic reaction which comprises stimulation of cellular overall RNA and protein synthesis (5-7)

and S-phase (1,2). In lytic infection of monkey kidney or CV-1 cultures (a monkey kidney cell line) S-phase is paralleled by the replication of SV40 DNA, the production of progeny virus and is followed by cell lysis. In transforming (abortive) infection of primary mouse kidney cultures, which are nonpermissive for SV40, S-phase is followed by mitosis without viral DNA replication (1-3, 8,9).

Viable mutants with deletions in the intron of large T-antigen, referred to as SV40 dl 54-59, direct synthesis of large T-antigen while either no small T-antigen or only truncated forms are synthesized (10-13). SV40 dl 54-59 mutants induce tumors in hamsters, however, after a longer latency than wt SV40 (14-16); in CV-1 cultures they lead to a lytic infection with a lower yield of infective progeny virus than wt SV40 (10). Although SV40 dl 54-59 mutants transform nonpermissive cells in vitro, their transforming ability is decreased to an extent which markedly depends on the cell type, the physiological state of the cultures (resting or proliferative) at the time of infection and the transformation assay used (10-12,17-20).

To obtain information on the functional roles of SV40 large and small T-antigen, respectively, in the induction of the mitotic reaction we compared lytic and transforming infection induced either by wt SV40 or the SV40 54-59 deletion mutants 883 and 891 (10). Mutant dl883 is deleted from 0.541-0.552 (57 bp; ref. 21,22) and synthesizes no small T-antigen (23). Mutant dl891, deleted from 0.590-0.595 (25bp; ref. 21,22), directs synthesis of a truncated small T-antigen (128 aa; Mr 11000) which comprises the 109 N-terminal amino acids of the normal reading frame and 19 C-terminal amino acids resulting from a frame shift (23); this truncated form is produced in lower amounts and is metabolically less stable than wt small T-antigen (11,23).

MATERIAL AND METHODS

Confluent secondary monkey kidney cell cultures (10-12 $x10^6$ cells /dish) or superconfluent CV-1 cultures (about 20 $x10^6$ cells /dish) were infected either with wt SV40 or the SV40

mutants d1883 and d1891 (25-50 PFU/cell) and incubated in medium containing 5% calf serum (Gibco) (7,24). All SV40 lysates had been obtained from CV-1 cultures infected at very low input multiplicities (0.01 PFU/cell; ref. 9) and their plaque forming titers were assayed on CV-1 cultures. Presence and localisation of the deletions in d1883 or d1891 were verified by digestion of purified SV40 DNA I with the restriction enzymes Taq I or Hind II + Hind III, followed by electrophoresis in polyacrylamide gels. All experimental points included mock-infected controls. If used, cytosine arabinoside (ara C 20 µg/ml; Sigma) was added to the culture medium immediately after virus adsorption (5). For all experimental points two dishes were used, unless indicated. All results reported here are representative of at least 3 independent experiments. The percentage of T-antigen containing nuclei was determined by the immunofluorescence reaction using antisera from hamsters bearing tumors induced by the inoculation of SV40-transformed hamster cells (8). To measure apparent rates of SV40 T-antigen synthesis, SV40- and mock-infected cultures were pulse-labeled for 1 hour-periods with 25-50 µCi/ml [^{35}S]methionine (25), lysed and the T-antigens isolated by immunoaffinity chromatography using antisera from tumor bearing hamsters (25). To obtain quantitative recovery of the T-antigens, the lysates were subjected to three successive cycles of immunoaffinity chromatography; the eluates from each cycle were individually subjected to SDS-PAGE in 7.5%-15% acrylamide linear gradient slab gels (26). Large T-antigen was revealed by staining the gels with Coomassie blue and by autoradiography; it was quantitated by scanning the gels at 630 nm, using bovine serum albumin as standard. To measure radioactivity, gel strips containing bands of large T-antigen were excised, rehydrated, discolored in 100 µl 30% oxygen peroxyde, dehydrated at 60°C and then disolved in 15% Soluene 100 (Packard), 21% NH_3. The values reported in Fig. 2 A and B are the sums of three cycles of immunoaffinity chromatography. Total cellular DNA, RNA and protein were extracted and quantitated colorimetrically under the conditions used earlier (7). To determine the percentage of DNA synthesizing cells,

mock- and SV40-infected cultures were pulse-labeled with [^{3}H]dT (5µCi/ml, 45 Ci/mmole; Amersham) for 1 hour-periods and then subjected to autoradiography (27). To measure apparent rates of synthesis and accumulation of SV40 DNA, three cultures per experimental point were pulse-labeled for 1 hour-periods with 25 µCi/ml [^{3}H]dT. Viral DNA was isolated according to Hirt (28), extracted with phenol and precipitated with ethanol. SV40 DNA I was purified by EtBr-CsCl equilibrium density gradient centrifugation; the fractions containing SV40 DNA I were pooled, EtBr removed with butanol, the DNA precipitated twice with ethanol and radioactivity and absorbance at 260nm (A260) were measured.

RESULTS

Lytic infection induced by wt SV40 and the SV40 mutants dl883 and dl891.

1) Synthesis and accumulation of SV40 large T-antigen

In monkey kidney cultures infected with wt SV40, dl883 or dl891, T-antigen could be detected by the immunofluorescence reaction in 1% of the nuclei by 8-9 hours, in 50-80% by 16 hours and in virtually 100% of the nuclei by 24 hours after infection. Throughout the experiments intensity and distribution of the nuclear immunofluorescence were indistinguishable in wt SV40- and dl mutant-infected cells.

SV40- and mock-infected cultures were pulse-labeled with [^{35}S]methionine and the T-antigens isolated and purified by immunochromatography; judged by autoradiography of the gels and by measurement of radioactivity in excised gels strips, synthesis of large T-antigen began 5-6 hours after infection with wt SV40 or the dl mutants and reached a peak around 18 hours (Fig. 1 A). In cultures infected with wt SV40 synthesis of large T-antigen reached a second peak around 36 hours which was not observed after infection with the dl mutants (Fig. 1 A).

Staining of the gels with Coomassie blue revealed a faint band of large T-antigen by 10-12 hours after infection with wt SV40 or the dl mutants; a maximum plateau was reached by

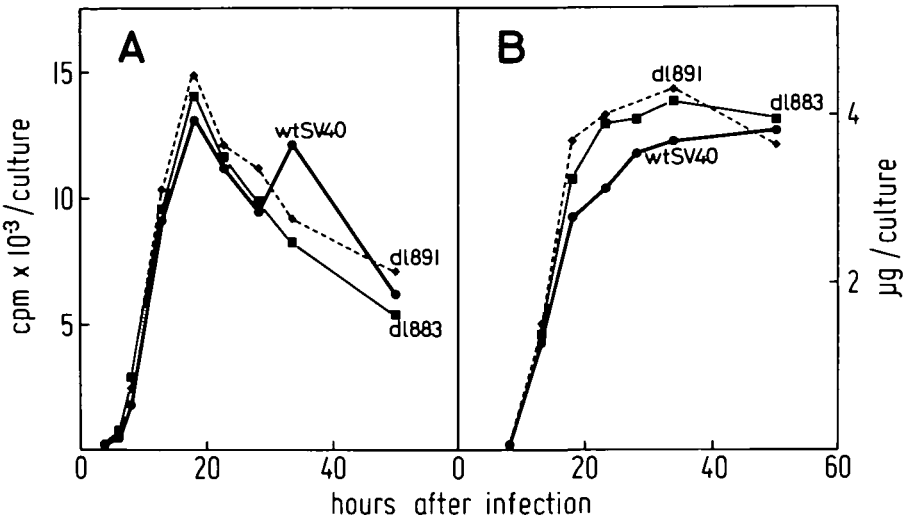

Figure 1
Synthesis (A) and accumulation (B) of SV40 large T-antigen in monkey kidney cultures infected with wt SV40, dl883 or dl891. SV40- and mock-infected (not shown) cultures were pulse-labeled for 1 hour-periods with [^{35}S]methionine at different times between 3 and 50 hours. The experimental points are the sums of 3 cycles of immunoaffinity chromatography (see Methods). The values in Fig. 1A were obtained by measuring radioactivity in excised gel strips and those in Fig. 1B by scanning the Coomassie blue-stained gels at 630 nm (see Methods).

30-40 hours (Fig. 1 B) when wt SV40- and dl mutant-infected cultures contained 3-4 µg/dish of large T-antigen.

Figure 2 shows the autoradiographs of gels containing T-antigens isolated by a single (first) cycle of immunoaffinity chromatography; the cultures had been pulse-labeled with [^{35}S]methionine from 17 to 18 hours, when T-antigen synthesis was at its maximum (Fig. 1 A). Under the conditions used 80% and 50-60% of large and small T-antigen, respectively, were recovered during the first cycle and most of the remainder during the second cycle of immunoaffinity chromatography. The bands of large T-antigen produced by SV40- or the mutant-infected cultures were indistinguishable, determined by staining the gels with Coomassie blue (Fig. 1B), by autoradiography of the gels (Fig 2) or by radioactivity measurements (Fig. 1A). In extracts from wt SV40-infected

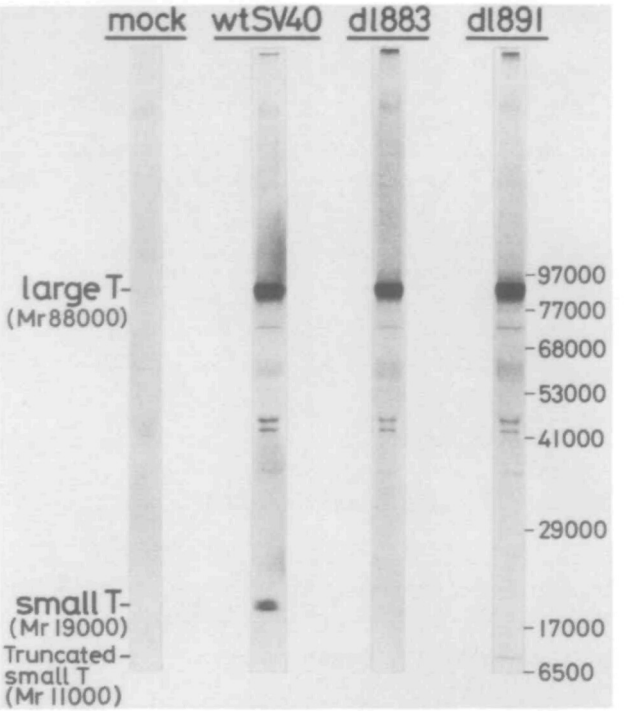

Figure 2
T-antigens synthesized in monkey kidney cultures infected with wt SV40, dl883 or dl 891. SV40- and mock-infected cultures were pulse-labeled with [^{35}S]methionine from 17-18 hours when rate of T-antigen synthesis was at its maximum (Fig.1). The T-antigens from the first cycle of immunoaffinity chromatography (see Methods) were subjected to SDS-PAGE in linear gradient gels (7.5-15% acrylamide). The gels were stained with Coomassie blue (not shown) and then subjected to autoradiography. The molecular weight markers are the same as used previously (7).

cultures small T-antigen could easily be detected by staining with Coomassie blue (data not shown), by autoradiography of gels (Fig. 2) or by radioactivity measurements (Fig. 1 A). As expected (23), extracts from dl891-infected cultures contained trace amounts of truncated small T-antigen (Mr 11000), detectable by autoradiography only (Fig. 2) while in extracts from dl883-infected cultures no small T-antigen was found.

In CV-1 cultures synthesis and accumulation of SV40 large T-antigen (maximum 6-7 µg/dish by 30 hrs) were

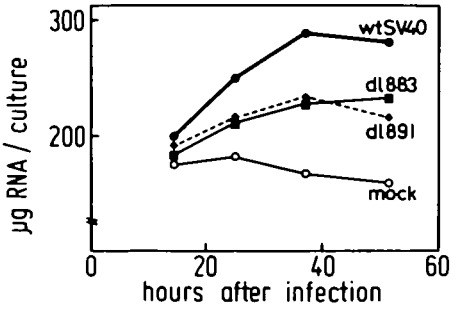

Figure 3
RNA content, determined colorimetrically (see Methods) of monkey kidney cultures infected with wt SV40, dl883, dl891 or mock-infected.

indistinguishable after infection with wt SV40 or the dl mutants (data not shown) whereas in cultures infected with the dl mutants only trace amounts of truncated small T-antigen (M_r 11000; dl891) or no small T-antigen (dl 883), were found (31 and unpublished observations).

2) The SV40-induced increase in cellular RNA and protein.

In monkey kidney cultures infected with wt SV40 or the dl mutants the amounts of total RNA and protein began to increase around 15 hours and reached a maximum plateau by 35-40 hours; at this time wt SV40-infected cultures contained about 45% (Fig. 3,4 and ref. 7) and dl mutant-infected cultures 20-25% more RNA and protein than mock-infected controls (Fig. 3,4). The kinetics of the increase was indistinguishable whether infection took place in absence or presence of ara C (ref. 7 and unpublished results). Nuclear and cytoplasmic fractions obtained from mock-, wt SV40- or dl mutant-infected monkey kidney cultures always contained 30% and 70%, respectively, of total cellular RNA and protein. Throughout infection with wt SV40 or the dl mutants this ratio was maintained and the

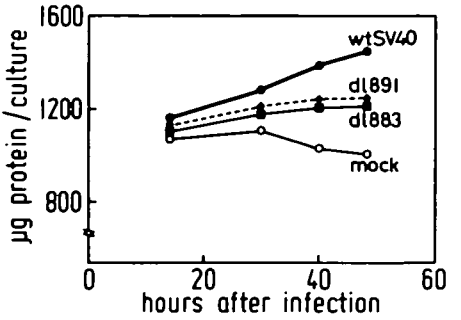

Figure 4
Protein content, determined colorimetrically (see Methods), of monkey kidney cultures infected with wt SV40, dl883, dl891 or mock-infected.

kinetics of the increase in nuclear and cytoplasmic RNA and protein corresponded to that observed in unfractionated cultures (data not shown). Cultures infected with wt SV40 or the dl mutants were pulse-labeled with [^{3}H]uridine for 1 or 3 hour-periods at different times between 12 and 55 hours; nuclear and cytoplasmic RNA was extracted with phenol (29) and analysed by gel electrophoresis (30). The results showed that infection with wt SV40 or the dl mutants led to stimulated synthesis of all major RNA species and that the increase in nuclear and cytoplasmic RNA, measured by A260, mainly reflected the accumulation of 18S and 28S rRNA (data not shown).

In CV-1 cultures infected with wt SV40 ($\pm$araC) or the dl mutants, total cellular RNA and protein began to increase around 20 hours after infection; a maximum plateau was reached by 60-70 hours when cultures infected with wt SV40 contained 60-70% (7) and those infected with the dl mutants 30-35% more RNA and protein than mock-infected controls.

3) SV40-induced synthesis of cellular and viral DNA.

Cytofluorometry and biochemical determinations of total DNA showed that confluent secondary monkey kidney cultures consisted mainly of Go/G1-arrested epithelial cells (5); this pattern did not detectably change during the experimental period (up to 7 days) and was independant of the presence (5% or 10%) or absence of bovine serum in the culture medium. Infection with wtSV40 induced within 45 hours in $>$ 80% of the cells duplication of the cellular DNA (S-phase) and the production of SV40 DNA, followed by cell lysis. Infection with SV40 tsA58, a temperature-sensitive mutant, unable to initiate SV40 viral DNA replication at 41°C, induced at 41°C S-phase followed by mitosis. The induction of a mitotic reaction with wt SV40 or tsA58 was independant of the presence or the absence of serum in the culture medium.

Mock-infected monkey kidney cultures contained 2-3% DNA synthesizing cells, determined by pulse-labeling with [^{3}H]dT for 1 hr-periods, followed by autoradiography (ref. 5 and Fig. 5). In cultures infected with wt SV40 or the dl mutants the number of DNA synthesizing cells began to increase by 15-20 hours; it reached a maximum plateau around 35 hours when in wt

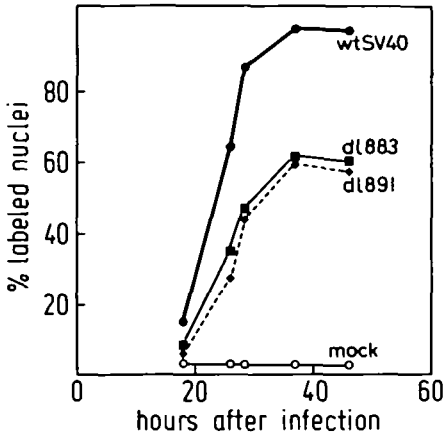

Figure 5
Percentage of DNA synthesizing cells in monkey kidney cultures infected with wt SV40, dl883, dl891 or mock-infected; cultures were pulse-labeled with [^{3}H]dT for 1 hour-periods at different times between 16 and 46 hours and then subjected to autoradiography (see Methods).

SV40-infected cultures $>$ 98% (5) and in dl mutant-infected cultures 50-60% of the cells were engaged in DNA synthesis (Fig. 5).

Determined colorimetrically, DNA content increased around 20 hours after infection with wt SV40 or the dl mutants and approached a maximum around 50 hours; at this time wt SV40-infected cultures contained about 2.3 times and dl mutant-infected cultures 1.5-1.6 times more DNA than mock-infected controls (Fig. 6).

Cultures were pulse-labeled with [^{3}H]dT at different times after infection, SV40 DNA I was selectively extracted and purified by EtBr-CsCl equilibrium density centrifugation. Synthesis of SV40 DNA I (wt SV40, dl883 or dl891) began by 15-20 hours, determined by radioactivity measurements. SV40 DNA I (wt, dl883 and dl891) could be detected by A260 at the

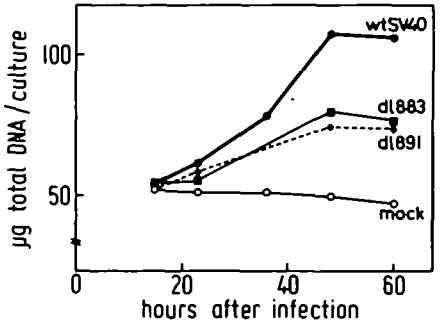

Figure 6
Total (i.e. cellular and viral) DNA content of monkey kidney cultures infected with wt SV40, dl883, dl891 or mock-infected was determined colorimetrically (see Methods).

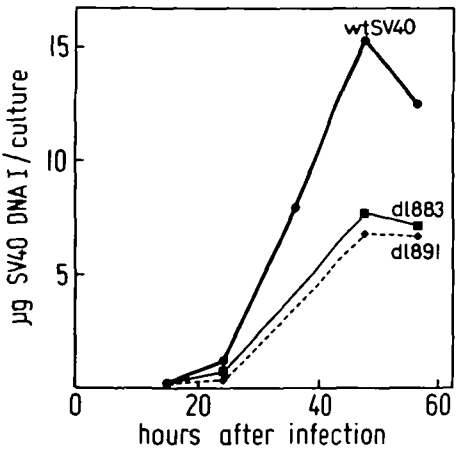

Figure 7
SV40 DNA I was isolated from monkey kidney cultures infected with wt SV40, dl883 or dl891 and quantitated by measuring A260. No DNA could be detected at the position of SV40 DNA I either by A260 (limit of detection 0.25 µg) or radioactivity measurements in EtBr-CsCl gradients with extracts from mock-infected cultures (data not shown).

earliest around 24 hours (0.5-1 µg/dish); the amounts of SV40 DNA I and apparent rate of synthesis reached a maximum around 50 hours when wt SV40-infected cultures contained 15-18 µg and dl mutant-infected cultures 7-8 µg per dish.

In mock-infected CV-1 cultures 2-3% of the cells synthesized DNA, determined by autoradiography (7). In cultures infected with wt SV40 or the dl mutants the number of DNA synthesizing cells began to increase around 20 hours; it reached a maximum around 50 hours when in wt SV40-infected cultures $>$98% of the cells synthesized DNA (7) but only 50-60% in dl mutant-infected cultures.

Synthesis of SV40 DNA I (wt, dl883 and dl891) began around 24 hours, determined by radioactivity measurements, and SV40 DNA could be detected by A260 (0.5-1µg /dish) at the earliest around 36 hours; the amounts of SV40 DNA I and apparent rate of viral DNA synthesis approached a maximum around 70 hours when CV-1 cultures infected with wt SV40 contained 30-35 µg and cultures infected with the dl mutants 15-18 µg per dish (32 and unpublished results).

DISCUSSION

During lytic infection of quiescent monkey kidney and CV-1 cell cultures with wt SV40 or the SV40 dl mutants 883 and 891 the kinetics of synthesis and accumulation of SV40 large T-antigen was very similar, leading to a maximum of 0.3-0.4 pg

of large T-antigen per cell. Virtually 100% of the monkey kidney and CV-1 cells infected with wt SV40 or the dl mutants participated in T-antigen synthesis, judged by the immunofluorescence reaction. Judged by autoradiography, wtSV40 induced in >98% and the dl mutants in 50-60% of the cells DNA synthesis. Whereas both wtSV40 and the dl mutants stimulated overall cellular RNA and protein synthesis, the maximum increase in dl mutant-infected cultures of total RNA and protein was only 50-60% of that observed in wt SV40-infected cultures. Similarly, the maximum increase in total cellular DNA and the amounts of SV40 progeny DNA in dl mutant-infected cultures corresponded only to about half the values measured in wtSV40-infected cultures. These results are thus compatible with the assumption that wtSV40 induced in >98% and the dl mutants in 50-60% of monkey kidney and CV-1 cultures a mitotic reaction and the production of SV40 progeny DNA.

In a parallel study we compared transforming (abortive) infection induced by wt SV40 (8,9) or the dl mutants 883 and 891 in G_0-arrested primary mouse kidney cell cultures. The results (ref. 31 and unpublished observations) revealed the same phenomenon as observed in lytic infection: the kinetics of synthesis and accumulation of large T-antigen was indistinguishable in wt- and dl mutant-infected cultures (0.4-0.5 pg/cell); in wt SV40-infected cultures T-antigen synthesis was followed by a complete mitotic cycle. In parallel cultures infected with the dl mutants 50-60% of the T-antigen synthesizing cells underwent a mitotic reaction while in the remainder no effect on DNA, RNA or protein synthesis was noted (not shown).

Synthesis of SV40 large T-antigen alone is thus sufficient to induce in 50-60% of the infected monkey kidney, CV-1 and mouse kidney cells a mitotic reaction and, in permissive cells, the production of viral progeny DNA whereas in the remainder of the cells apparently also synthesis of wt small T-antigen is required. This suggests that wt small T-antigen exerts in 40-50% of the cells a helper function prerequisite for the induction of the mitotic reaction and, in permissive cells, for the production of viral progeny DNA. Recent results indicate

that polyoma small and middle T-antigens play a helper function in the replication of polyoma viral DNA (32).

The mitotic reaction induced by SV40 and polyoma virus is similar to that triggered by nonviral mitogens in T-lymphocytes and in mouse 3T3 fibroblasts (ref. 33-34). The observation that a single viral protein, SV40 large T-antigen, is able to trigger in growth-arrested cells a mitotic reaction, points therefore to the possibility that the conversion of uninfected mammalian cells from a quiescent to a proliferating state may be under the control of one or a small number of cellular genes encoding proteins functionally analogous to SV40 large T-antigen (33).

ACKNOWLEDGEMENTS

We are grateful to Drs. E.W. Khandjian, M. Schwyzer and H. Türler for useful discussions and suggestions. We thank Mr. Bensemmane for preparing the cultures, Mrs. Y. Delotto and Mr. O. Jenni for preparing the figures. This work was supported by grant No 3.072.81 from the Swiss National Science Foundation and grants from the Sandoz Research Foundation.

REFERENCES

1. Weil, R. (1978). Biochim. Biophys. Acta 516, 301-388.
2. Acheson, N.H. (1980). In Molecular Biology of Tumor Viruses, part 2, (Tooze, J. ed.), pp. 125-204, Cold Spring Harbor Laboratory, New York.
3. Griffin, B.E. (1980). In Molecular Biology of Tumor Viruses, part 2, (Tooze, J. ed.), pp. 61-123, Cold Spring Harbor Laboratory, New York.
4. Berk, A.J. & Sharp, P.A. (1978). Proc. Natl. Acad. Sci. USA 75, 1274-1278.
5. Weil, R., Türler, H., Léonard, N. & Ahmad-Zadeh, C. (1977). INSERM 69, 263-280.
6. Baserga, R., Ide, T. & Whelly, S. (1977). Cold Spring Harbor Symp. Quant. Biol. 42, 685-691.
7. Khandjian, E.W., Matter, J.-M., Léonard, N. & Weil, R. (1980). Proc. Natl. Acad. Sci. USA 77, 1476-1480.
8. May, E., May, P. & Weil, R. (1971). Proc. Natl. Acad. Sci. USA 68, 1208-1211.
9. May, E., May, P. & Weil, R. (1973). Proc. Natl. Acad. Sci. USA 70, 1654-1658.
10. Shenk, T.E., Carbon, J. & Berg, P. (1976). J. Virol. 18, 664-671.
11. Sleigh, M.J., Topp, W.C., Hanich, R. & Sambrook, J.F. (1978). Cell 14, 79-88.
12. Feunteun, J., Kress, M., Gardes, M. & Monier, R. (1978). Proc. Natl. Acad. Sci. USA 75, 4455-4459.

13. Pipas, J.M., Adler, S.P., Peden, K.W.C. & Nathans, D. (1979). Cold Spring Harbor Symp. Quant. Biol. 39, 285-291.
14. Lewis, A.M. & Martin, R.G. (1979). Proc. Natl. Acad. Sci. USA 76, 4299-4302.
15. Topp, W.C., Rifkin, D.B. & Sleigh, M.J. (1981). Virology 111, 341-350.
16. Dixon, K., Ryder, B.-J. & Burch-Jaffe, E., (1982). Nature 296, 672-675.
17. Bouck, N., Beales, N., Shenk, T., Berg, P. & DiMayorca, G. (1978). Proc. Natl. Acad. Sci. USA 75, 2473-2477.
18. Martin, R.G., Setlow, V.P., Edwards, C.A.F. & Vembu, D. (1979). Cell 17, 635-643.
19. Martin, R.G., Setlow, V.P. & Edwards, C.A.F. (1979). J. Virol. 31, 596-607.
20. Friske, R.J., Rifkin, D.B. & Topp, W.C. (1979). Cold Spring Harbor Symp. Quant. Biol. 39, 325-331.
21. Thimmappaya, B. & Shenk, T. (1979). J. Virol. 30, 668-673.
22. Volckaert, G., Feunteun, J., Crawford, L.V.,Berg, P. & Fiers, W. (1979). J. Virol. 30, 674-682.
23. Khoury, G., Gruss, P., Dhar, R. & Lai, C.-J. (1979). Cell 18, 85-92.
24. Ahmad-Zadeh, C., Allet, B., Greenblatt, J. & Weil, R. (1976). Proc. Natl. Sci. USA 73, 1097-1101.
25. Schwyzer, M., Weil, R., Frank, G. & Zuber, H. (1980). J. Biol. Chem. 225, 5627-5634.
26. Laemmli, U.K. (1970). Nature 227, 680-685.
27. Matter, J.-M., Khandjian, E.W., Weil, R. (1983). Nucleic Acids Res. 11, 1039-1058.
28. Hirt, B. (1967). J. Mol. Biol. 26, 365-369.
29. Scherrer, K. (1969). In Fundamental Techniques in Virology (Habel, K. & Salzman, N. P. eds), pp. 413-432.
30. Peacock, A.C. & Dingman, C.W. (1968). Biochemistry 7, 668-674.
31. Gauchat, J.-F. (1985). PhD Thesis, University of Geneva, Switzerland.
32. Templeton, D., Simon, S. & Eckhardt, W. (1986). J. Virology 57, 367-370.
33. Matter, J.-M., Tiercy, J.-M. and Weil, R. (1983). Nucleic Acids Res. 11, 6611-6629.
34. Tiercy, J.-M. and Weil, R. (1983). Eur. J. Biochem. 131, 47-55.