JOURNAL OF VIROLOGY, Jan. 1975, p. 145-150
Vol. 15, No. 1

Printed in U.S.A.

DNA Infectivity and the Induction of Host DNA Synthesis with Temperature-Sensitive Mutants of Simian Virus 40

JANICE YANG CHOU AND ROBERT G. MARTIN*
Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20014

Received for publication 13 August 1974

Host DNA synthesis is induced when CV-1 (monkey kidney) cell cultures are infected at 40 C with wild-type virions or with temperature-sensitive Simian virus 40 mutants of the "early" complementation group *A*. Host DNA synthesis is not induced when cultures are infected with mutants of the late complementation group *D*. The simplest explanation for these observations, that induction depends not upon the expression of some early gene function but rather on the presence of an active *D* protein in the infecting virion, has been examined. Indirect experiments suggest that this explanation is not correct. Moreover, the induction of host DNA synthesis is impaired when cultures are infected with mutants of the *A* group at 42.5 C rather than 40 C, suggesting that the *A* function may be responsible for host induction. The inability of *D* virions to induce host DNA synthesis may reflect their inability to "uncoat" at 40 C.

It has been proposed that a member of the *D* complementation group of Simian virus 40 (SV40), *D101*, is defective in its ability to "uncoat" at the restrictive temperature; *D101* virions adsorb and penetrate normally but do not induce any early viral function, or host or viral DNA synthesis, whereas *D101* DNA is infectious for one round of replication (15). These results, in conjunction with the conclusions of Lai and Nathans (10) that *D* mutations map in the "late" region, have led to the proposal (4, 15) that the *D* cistron encodes a virion protein which remains associated with the viral DNA when *D* virions infect permissive cells at 40 C. An analysis of the products of complementation between *D* mutants, wild-type, and other temperature-sensitive mutants are consistent with this hypothesis (4). That the failure to uncoat is a general property of *D* mutants is now suggested by the demonstration that DNA from all *D* mutants is infectious for a single cycle of viral replication.

The induction of host DNA synthesis by SV40 in permissive cells (considered in the second section of this paper) is poorly understood. Kinetic analyses have demonstrated that the induction starts at about the same time as the onset of viral DNA replication (7, 8). It is therefore impossible to determine from the kinetics whether host induction is a consequence of an "early" or a "late" viral function. That induction is seen in nonpermissive cells which do not express late function suggests that an early function is required (8, 16). If one assumes that the same viral function is required for the induction of host DNA synthesis in CV-1 and 3T3 cells a conflict still remains since 3T3 cells transformed by *A* mutants (*A* is the only known early function) have been reported to maintain the transformed phenotype (host DNA induction) at the restricting temperature (18). This, and the fact that mutants of the *A* group induce host DNA synthesis in CV-1 cells although they do not replicate viral DNA at 40 C (5, 18) suggested either that an early function other than *A* was responsible for induction (since *A* mutants do not express late functions), or that induction was a consequence of a viral component introduced by the virion upon infection. Since mutants of the *D* group neither induce host DNA synthesis nor replicate viral DNA at 40 C (5) the latter hypothesis seemed worth reexamining. Arguing against this possibility was the observation that nitrous acid treatment of virions destroyed their ability to induce (8). Experiments to test these possibilities have been negative. However, we have found that certain *A* mutants have a reduced capacity for host induction at 42.5 C even though they are normal at 40 C. This suggests that *A* function is pleiotropic, i.e., it is both required for the initiation of viral DNA synthesis (5, 18) and the induction of host DNA synthesis. Why our mutants are more temperature sensitive for replication than for induction, remains unclear.

MATERIALS AND METHODS

Virus and cells. The virus stocks, mutants, and CV-1 cells used have been described (3). The primary AGMK cells were supplied by Flow Laboratories and the BSC1 cells kindly provided by Donald LeBlanc and tested by him to verify that no induction of host DNA synthesis was observed after SV40 infection (Fig. 1 of reference 1).

Media and plaque assays. The media and plaque assays have been described (3, 14). Depleted medium was the modified Eagle medium supplemented with 5% fetal bovine serum (3E5) removed after 4 to 5 days over confluent CV-1 cells.

DNA infections. DNA was prepared by infecting confluent monolayers of CV-1 cells in 75 cm^2-flasks at an MOI of approximately 5 PFU/cell and after 72 h of incubation at 33 C the medium was removed, the cells were washed twice with phosphate-buffered saline (PBS) (14), and lysed by the addition of 1 ml per flask of a solution containing 0.6% sodium dodecyl sulfate, 0.01 M EDTA, and 0.01 M Tris, pH 7.6 (lysing solution). After adjusting the extracts to 1 M NaCl and incubation overnight at 4 C, the extracts were clarified by centrifugation (9) and the supernatant solutions were dialyzed overnight against 0.01 M Tris, pH 7.5, 1 M NaCl, and 1 mM EDTA. The resulting DNA extracts had absorbancies at 260 nm of approximately 1.

The infectivity of viral DNA was assayed by a slight modification of published techniques (12, 15). The medium was aspirated from confluent monolayers of cells in 25 cm^2-flasks, or in 2 cm^2-wells, and treated with 0.5 ml or 0.2 ml, respectively, of a solution prepared as follows: to 0.1 ml of the DNA extract was added 0.1 ml of a 4 mg/ml DEAE dextran suspension (Pharmacia, lot no. 1471, molecular weight $\sim 2 \times 10^6$, suspended in 0.15 M Tris, pH 7.5, and sterilized by autoclaving) and 0.8 ml of modified Eagle medium without serum supplement (3EO). After allowing 30 min for adsorption at 40 or 33 C, the cells were washed twice with PBS and 1 ml fresh or depleted 3E5 medium was added. Samples were incubated for 3 days at 40 C or 6 days at 33 C and frozen. The samples were thawed, refrozen and rethawed, and titered for plaque-forming ability at 33 C. Individual plaques were picked (3) and assayed for the ability of the virus to grow at 33 and 40 C.

Co-infection by viral DNA and virions. For co-infection experiments, DNA extracts prepared as above were diluted with DEAE dextran and 3EO as above. Confluent monolayers of CV-1 cells in 25 cm^2-flasks received 0.25 ml of DNA solution or DEAE dextran solution without DNA and 0.25 ml of mock lysate or virus suspensions containing 2×10^6 PFU/ml. After 45 min for adsorption at 40 C, the suspensions were removed, the cells washed twice with PBS, and then incubated in 3EO for 40 h at 40 C. The medium was aspirated and the cells were labeled by incubation for 1 h with 1.5 ml of 3EO containing [^{3}H]thymidine, 100 μCi/ml. Extracts were prepared and separated into supernatant (viral DNA) and precipitant (host DNA) fractions by the method of Hirt (9). The DNA was precipitated by the addition of cold 10% trichloroacetic acid. Samples were collected on glass filters, washed with cold 5% trichloroacetic acid and 95% ethanol, dried, and the radioactivity was determined in a Mark II scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.).

UV irradiation and assay for host induction. A stock of mutant *A209* virions (3.6×10^8 PFU/ml) was diluted fivefold into PBS (to dilute the UV absorbing material in the medium). The bottom of a 9-cm (diameter) glass petri plate containing 30 ml of this suspension was mechanically agitated while being exposed to a 15-W germicidal lamp (General Electric) 20 cm above the liquid surface (output approximately 10^4 ergs/cm^2 per s). Portions of 5 ml were removed at 0, 2.5, 5, 10, and 20 min of exposure and titered.

The samples were tested for their ability to induce host DNA synthesis by infecting monolayers with a constant volume of the irradiated suspensions (MOI = 1.4 PFU/cell for the unirradiated material). After aspiration of the medium, confluent monolayers in 2-cm^2 wells of the 24-well plates of Linbro (New Haven, Conn.) were treated with 0.2 ml of UV-irradiated samples. Various dilutions of the samples in PBS were also prepared and used to infect other monolayers. After 2 h of adsorption at 40 C the virus suspensions were removed, 1 ml of 3EO was added, and the cultures were incubated for 26 h at 40 C. The cultures were labeled with 0.3 ml of medium with [^{3}H]thymidine as above. After incubation the monolayers were washed twice with PBS and lysed with 0.4 ml of lysing solution. The entire sample was transferred to a test tube with a Pasteur pipette, the well was rinsed with 0.2 ml of lysing solution and added to the test tube, and trichloroacetic acid was added as above. The precipitates were washed and counted as above.

Induction of host DNA synthesis by virions at 42.5 C. Confluent CV-1 monolayers in the 24-well Linbro plates were treated as above with various mutants at MOI = 4. The virus was adsorbed for 2 h and the cultures were then incubated in 3EO for 26 h at 42.5 C or 50 h at 33 C. The cells were radioactively labeled as above and after washing twice with PBS, 0.4 ml of lysing solution was added. Polyallomer centrifuge tubes (3 by 5/8 inches [7.62 by 1.59 cm]) (Beckman Instrument Co) were inverted and forced into the wells of the Linbro plates. The plates and tubes were then inverted to allow the extracts to drain into the tubes. The extracts were brought to 1 M in NaCl, fractions were separated according to the procedure of Hirt (9), and precipitated and counted as above. Each value at 33 C is the average of six cultures and at 42.5 C is the average of eight cultures.

RESULTS

Infectivity of viral DNA. DNA was prepared from representative strains of each mutant class and its infectivity was tested at permissive and restrictive temperatures. The results (Table 1) confirm that *D* mutant DNA is infectious at the restrictive temperature in CV-1, AGMK, and BSC1 cells. The number of progeny after a single round of infection with *D* mutant DNA was the same at both temperatures. DNA from mutants of the *A*, *B*, *C*, and *BC* classes was considerably

TABLE 1. *Infectivity of viral DNA from temperature-sensitive mutants of SV40*

Strain	CV-1 cells				BSC1 cells		Primary AGMK cells	
	Fresh medium		Depleted medium		Virus yield in PFU/ml		Virus yield in PFU/ml	
	Virus yield in PFU/ml		Virus yield in PFU/ml					
	40 C	33 C	40 C	33 C	40 C	33 C	40 C	33 C
Wild-type	4×10^5	6×10^5	8×10^4	8×10^4	1.6×10^5	$\sim 5 \times 10^5$	1.6×10^4	10^5
A207	20	6×10^5						
A209	0	1.6×10^5	<2	8×10^3	<2	8×10^4	<2	2×10^4
A239	0	$>6 \times 10^5$						
A241	0	$>6 \times 10^5$						
A255			<2	2×10^4				
A276	0	4×10^5						
B201	2.4×10^2	$>6 \times 10^5$	8×10^2	4×10^4	<2	$\sim 5 \times 10^5$	40	9×10^4
B204	1.1×10^2	$>6 \times 10^5$	3×10^2	6×10^4				
C219	2	2×10^5	4	2×10^3	3×10^2	1.5×10^5	4	10^3
C240	46	$>6 \times 10^5$						
C244	1.5×10^2	4.6×10^4						
C259	3×10^3	4×10^5						
C260	3×10^2	1.3×10^5						
C261	7.6×10^2	2×10^5						
BC230	2	1.5×10^5	20	4×10^4				
BC245	28	$>6 \times 10^5$	30	10^5	<2	$\sim 5 \times 10^5$	<2	1.5×10^5
D101	2×10^5	6×10^5	4×10^4	1.3×10^5	10^5	3×10^5	5×10^3	9×10^4
D202	1.1×10^5	1.4×10^5			5×10^3	10^4		
D222	4×10^5	6×10^5	8×10^3	4×10^4			10^4	4×10^4
D238	4×10^5	6×10^5	10^4	8×10^4	4×10^4	1.2×10^5	10^4	3×10^4
D263	8.3×10^4	3×10^5	3×10^4	6×10^4	7×10^4	$\sim 5 \times 10^5$	8×10^3	2×10^4
D270	2×10^3	2.4×10^4			8×10^3	3×10^4		
D275	1.6×10^5	4×10^5	8×10^3	9×10^4	5×10^4	$\sim 4 \times 10^5$	10^4	5×10^4

less infectious at the restrictive than at the permissive temperature. Because it has been demonstrated that virion infections by *D* mutants are suppressed by fresh medium containing high serum concentrations (4), assays were also performed in depleted medium with similar results (Table 1).

The progeny of DNA infections at 40 C were still temperature sensitive.Two or three plaques of each mutant (grown at 33 C) obtained after the DNA infection at 40 C were tested for their ability to grow at both temperatures. Every plaque (20 from *D* mutants and 26 from all other mutant classes), except the two obtained from the cultures infected with wild-type DNA were temperature sensitive. We conclude that none of the preparations contained significant amounts of revertant wild-type DNA, and that *D* mutant DNA is infectious at 40 C yielding virions which are still temperature sensitive.

The observations: (i) that only a small percentage of monkey cells infected with excess SV40 viral DNA are capable of productive infection (12); (ii) that *D* mutant DNA is as infectious as wild-type DNA at the restrictive temperature for a single growth cycle; (iii) that fresh serum suppresses *D* virion infection (4); and (iv) that *D* mutant virion infection fails to induce host cellular DNA synthesis (5), suggested the following model: upon entering the cell the virion becomes "uncoated" passing through a stage in which the protein encoded by the *D* cistron remains associated with the viral DNA. The *D* protein (either free or associated with the viral genome) might also affect the host cell in such a way that the host would be stimulated to enter the S phase of growth which appears to be required for the initiation of viral DNA replication (13), i.e., host DNA synthesis would be induced by the *D* protein. All of the above observations would be accounted for by such a model. Both wild-type and *D* mutant DNA infections could only proceed in that small percentage of the cells which entered S phase soon after the DNA had reached the nucleus, explaining the low infectivity of DNA as compared with virions. Fresh serum would stimulate more cells to enter the S phase and hence suppress infections by *D* mutant virions. Mutants defective in the protein encoded by the *D* cistron would then fail to induce cellular DNA synthesis. The results of several experiments are inconsistent with this model.

Co-infection with A virions and viral DNA.

One prediction of the model is that *A* mutant virions (which induce host DNA synthesis without synthesizing their own viral DNA) should greatly enhance the infectivity of wild-type DNA at the restrictive temperature by providing good *D* protein. The percentage of productively infected cells in such co-infected cultures should increase relative to cultures infected with DNA alone. However, no enhancement of the rate of viral DNA synthesis by either wild-type or *D* mutant DNA was observed after 40 h at 40 C as a result of co-infection with *A* virions. The results (Table 2) suggest that host DNA synthesis is stimulated by *A209* virions alone (first row, last column) but not by *D202* virions (second row). Viral DNA replication was seen after infection with either wild-type or *D* mutant DNA, but the rate of viral DNA synthesis in the co-infected cultures was no greater than that observed after DNA infection alone (compare first data column rows 4 and 5 with rows 6 and 7). Thus, *D* protein does not enhance DNA infectivity. That co-infection had occurred is demonstrated by the fact that the cultures co-infected with *A* virions and *D* DNA synthesized viral DNA and were induced for host DNA synthesis (compare for example rows 1, 4, and 6).

TABLE 2. *Co-infection of CV1 cells by temperature-sensitive virions and viral DNA*

Infecting agent		Viral DNA synthesis (counts/min[a] $\times 10^{-3}$)	Induction of host[b] DNA synthesis
Virion	DNA		
A209		2.6	2.3
D202		3.4	1.4
	A209	0.6	1.0
	D202	20.9	1.7
	Wild type	29.1	2.4
A209	*D202*	17.1	2.7
A209	Wild type	31.5	4.0
D202	*A209*	2.0	1.1
D202	Wild type	25.2	1.8

[a] The numbers represent counts/min in the supernatant fraction (see Materials and Methods), with the background (4,000 counts/min) from a monolayer infected with 0.25 ml of mock lysate and 0.25 ml of the DEAE dextran solution in 3E0 subtracted.

[b] Host induction is the fold increase in the precipitate fractions (see Materials and Methods) over the mock-infected cultures (2,000 counts/min).

Induction of host DNA synthesis by UV-irradiated A mutant virions. If a structural component of the virion were responsible for the induction of host DNA synthesis then UV irradiation of virions should not result in a loss of the ability of those virions to induce. Although target-size measurements of host DNA induction (6, 8), T-antigen induction (17, 19), and transformation (1, 2, 17, 19), by SV40 or polyoma virions have been performed previously, two technicalities could invalidate the host DNA induction results. First, the experiments were performed at only high virus concentrations and without convincing demonstration in the case of SV40 (16) that induction was linearly related to the MOI. Indeed, one might expect that such experiments should only be performed at MOI ≤ 1. Secondly, DNA induction experiments were performed either in non-permissive cells (6), or in permissive X-irradiated cells where a significant percentage of the total radioactivity incorporated represents viral DNA replication (8). To overcome these complications host induction was measured in cells infected with irradiated *A209* virus at different MOI at the restrictive temperature. At 40 C, *A209* induces host DNA synthesis but not viral DNA (5, 18). The assays could therefore be performed by determining the total incorporation of thymidine into acid-precipitable material without fractionation. Figure 1 demonstrates that the induction of host DNA synthesis is linearly related to the MOI over the range used in these experiments (approximately 0.4 to 1.4).

UV irradiation of virions does inactivate their ability to induce host DNA synthesis under conditions where viral DNA synthesis is completely inhibited. In Fig. 2, virion viability and the slopes of the host DNA induction curves (of Fig. 1) are plotted as a function of the time of UV irradiation. The relative slopes of the two curves indicate that the loss of viability is 2.9 times more rapid than the loss of the ability to induce host DNA synthesis, i.e., that the target size for the induction of host DNA is 35% of the target size for viability. We conclude that a component of mature virions is not responsible for the induction of host DNA synthesis. Therefore, either (i) an early cistron other than *A*, approximately one-third of the viral genome, must exist and be translated for the induction of host DNA synthesis, or (ii) the *A* cistron is pleiotropic, serving both to initiate viral DNA synthesis and to stimulate host DNA synthesis. However, if the latter were true, at least some *A* mutants should be defective for the induction of host DNA synthesis.

Induction of host DNA synthesis by A mutants. We previously reported that the induction of host DNA synthesis was normal in *A* mutants at 40 C even though viral DNA synthesis was undetectable at this temperature. We therefore reexamined the induction of host

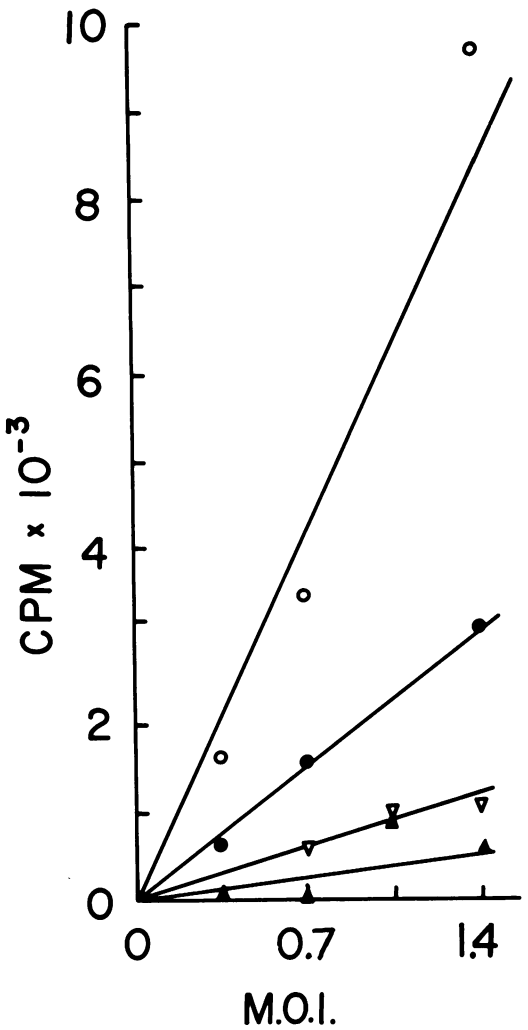

FIG. 1. *Induction of host DNA synthesis by A209 26 h postinfection at 40 C as a function of MOI. Samples were irradiated with UV and checked for their ability to induce host DNA synthesis in CV-1 cells (see Materials and Methods). The background from mock-infected cultures (2,970 counts/min) is subtracted. All points are the average of at least two cultures. Time of irradiation:* (○) *nonirradiated;* (●) *2.5 min of irradiation;* (▽) *5 min of irradiation and* (▲) *10 min of irradiation. Only the induction observed by virions irradiated for 10 min shows considerable scatter.*

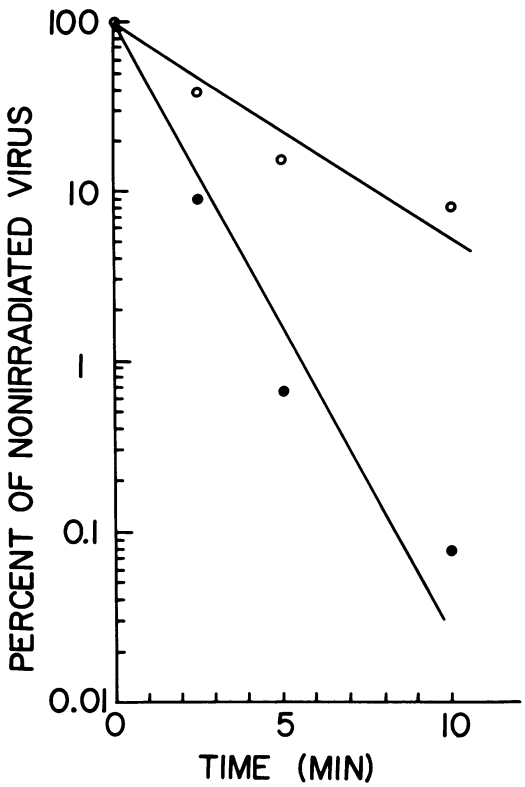

FIG. 2. *Relative UV target sizes for the induction of host DNA synthesis and virion viability. The slopes of the lines in Fig. 1 for the UV-irradiated versus nonirradiated virus are plotted versus time of irradiation* (○). *Virion viability is also plotted* (●). *The relative slopes of the two lines determines the relative target sizes for host induction and viability. This ratio remains similar if the 10-min point is eliminated.*

DNA synthesis at the highest temperature at which our cells remain viable, 42.5 C.

A mutants are partially temperature sensitive for the induction of host DNA synthesis. The ability to induce at 42.5 C was reduced in the *A* mutants (Table 3), though not completely abolished. In many experiments, *A255* appeared consistently to show the poorest ability to induce host DNA.

DISCUSSION

D mutant DNA is fully infectious at the restrictive temperature, suggesting that the expression of *D* function is not required for the production of infectious virions. Yet infection by *D* mutant virions does not result in viral growth. This apparent inconsistency can be fully explained by the hypothesis (4, 15) that the *D* cistron encoded protein is a part of mature virions which must be shed before transcription of the viral genome. In fact, early viral RNA is absent from cells infected with *D* mutants at 40 C (Saral, Khoury, Chou, and Martin, unpublished data; and Robb and Lopez, personal communication).

We have examined the possibility that the *D* protein might be related to the induction of

TABLE 3. *Induction of host DNA synthesis at 42.5 and 33 C by temperature-sensitive mutants of SV40*

Strain	Rate of cellular DNA synthesis		
	[a]Counts/min at 42.5 C × 10^{3a}	[a]Counts/min at 33 C × 10^{3a}	Ratio[b]
Mock	9.8	1.2	
Wild type	32.9	22.8	1.0
B204	24.3	12.9	1.2
BC230	16.6	10.6	0.7
D270	7.7	13.7	−0.2
A207	17.2	29.8	0.2
A209	18.6	27.6	0.3
A239	17.0	22.6	0.3
A241	14.8	25.2	0.2
A255	15.6	31.9	0.2
A276	14.4	24.9	0.2

[a] Numbers represent the counts/min in the precipitate fraction following the procedure of Hirt (9).

[b] Numbers represent (counts/min at 42.5 C − mock)/(Counts/min at 33 C − mock) and the ratios normalized to wild type = 1.

cellular DNA synthesis observed after virion infection. Co-infection of *A* virions with wild-type or *D* mutant DNA does not enhance viral replication. Furthermore, expression of some early function is required for host induction since UV-irradiated virions lose their ability to induce.

The conclusion was therefore reached either that an additional early function exists or that the *A* cistron is pleiotropic. To test the latter possibility which assumes that our mutants are more temperature sensitive for viral initiation than for host induction (reason unknown), a more careful examination of the induction of host DNA replication by *A* mutants was undertaken. This analysis showed that the *A* mutants are defective in the induction of cellular DNA synthesis at 42.5 C. We conclude that *A* is pleiotropic; it is required both for the initiation of viral DNA synthesis and for the induction of host DNA synthesis.

LITERATURE CITED

1. Basilico, C., and G. Di Mayorca. 1965. Radiation target-size of the lytic and the transforming ability of polyoma virus. Proc. Nat. Acad. Sci. U.S.A. **54**:125-127.
2. Benjamin, T. L. 1965. Relative target-sizes for the inactivation of transforming and reproductive ability of polyoma virus. Proc. Nat. Acad. Sci. U.S.A. **54**:121-124.
3. Chou, J. Y., and R. G. Martin. 1974. Complementation analysis of simian virus 40 mutants. J. Virol. **13**:1101-1109.
4. Chou, J. Y., and R. G. Martin. 1975. Products of complementation between temperature-sensitive mutants of simian virus 40. J. Virol. **15**:127-136.
5. Chou, J. Y., J. Avila, and R. G. Martin. 1974. Viral DNA synthesis in cells infected by temperature-sensitive mutants of simian virus 40. J. Virol. **14**:116-124.
6. Defendi, V., F. Jensen, and G. Sauer. 1967. Analysis of some viral functions related to neoplastic transformation, p. 645-663. *In* J. S. Colter and W. Paranchych (ed.), Molecular biology of viruses. Academic Press Inc., New York.
7. Dulbecco, R., C. H. Hartwell, and M. Vogt. 1965. Induction of cellular DNA synthesis by polyoma virus. Proc. Nat. Acad. Sci. U.S.A. **53**:403-410.
8. Gershon, D., P. Hausen, L. Sachs, and E. Winocour. 1965. On the mechanism of polyoma virus—induced synthesis of cellular DNA. Proc. Nat. Acad. Sci. U.S.A. **53**:1584-1592.
9. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365-369.
10. Lai, C.-Y., and D. Nathans. 1974. Mapping the genes of simian virus 40. Cold Spring Harbor Symp. Quant. Biol. Vol. 39.
11. LeBlanc, D. J., and M. F. Singer. 1974. Localization of replicating DNA of simian virus 40 in monkey kidney cells. Proc. Nat. Acad. Sci. U.S.A. **71**:2236-2240.
12. McCutchan, J. H., and J. S. Pagano. 1968. Enhancement of the infectivity of SV40 DNA with DEAE-dextran. J. Nat. Cancer Inst. **41**:351-357.
13. Pages, J., S. Manteuil, D. Stehelin, M. Fiszman, M. Marx, and M. Girard. 1973. Relationship between replication of SV40 DNA and specific events of the host cell cycle. J. Virol. **12**:99-107.
14. Robb, J. A., and R. G. Martin. 1970. Genetic analysis of simian virus 40: description of microtitration and replica plating techniques for virus. Virology **41**:751-760.
15. Robb, J. A., and R. G. Martin. 1972. Genetic analysis of simian virus 40. III. Characterization of a temperature-sensitive mutant blocked at an early stage of productive infection in monkey cells. J. Virol. **9**:956-168.
16. Sauer, G., and J. Defendi. 1966. Stimulation of DNA synthesis and complement-fixing antigen production by SV40 in human diploid cell cultures: evidence for "abortive" infection. Proc. Nat. Acad. Sci. U.S.A. **56**:452-457.
17. Seemayer, N. H., and V. Defendi. 1973. Analysis of minimal functions of simian virus 40. II. Enhancement of oncogenic transformation in vitro by UV irradiation. J. Virol. **12**:1265-1271.
18. Tegtmeyer, P. 1972. Simian virus 40 deoxyribonucleic acid synthesis: the viral replicon. J. Virol. **10**:591-598.
19. Yamamoto, H. 1970. Inactivation of the transforming capacity of SV40 and the oncogenicity of adenovirus 12 by ultraviolet irradiation. Jap. J. Microbiol. **14**:487-493.