JOURNAL OF VIROLOGY, Oct. 1972, p. 591-598
Vol. 10, No. 4

Simian Virus 40 Deoxyribonucleic Acid Synthesis: the Viral Replicon

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Received for publication 5 July 1972

Three temperature-sensitive (ts) mutants of simian virus 40 (SV40) in complementation group A (tsA7, tsA28, tsA30) have been isolated and characterized in permissive and restrictive host cells. At 41 C in the AH line of African green monkey kidney cells, the mutants are deficient in an early function required to produce infectious viral deoxyribonucleic acid (DNA). Temperature-shift experiments and analysis of SV40 viral DNA replication by gel electrophoresis have provided strong evidence that the ts gene product of the three mutants is directly required to initiate each new round of viral DNA replication but is not required to complete a cycle which has already begun. The synthesis of mutant DNA molecules themselves can be initiated by a nonmutant gene product in viral complementation studies at 41 C. The cell, however, cannot substitute a host function to provide the initiator required for the replication of free viral DNA. The viral initiator is also required to establish the stable transformation of 3T3 cells.

Simian virus 40 (SV40) may cause either productive infection of permissive cells (17) or transforming infection of certain restrictive cells (1, 21). Because of the small size of the SV40 genome (2), the viral gene(s) required for transformation may well also be essential for replication of the virus. This assumption has been the basis for the selection of mutants for the genetic analysis of the transformation process (18). The present communication describes a group of mutants whose temperature-sensitive (ts) gene product is required to initiate, but not to complete, a single round of viral deoxyribonucleic acid (DNA) replication in productive infection and is also required to initiate the stable transformation of restrictive cells.

MATERIALS AND METHODS

Cell culture. The AH line (5) of African green monkey kidney (AGMK) cells was cultivated in F12 medium containing 2 to 10% fetal calf serum (FCS). The mouse cell line 3T3 (21) was grown in F12 medium containing 10% FCS.

Virus. Wild-type (WT) SV40 was derived from the small-plaque strain of VA 45-54 by cloning as previously described (18). Mutants tsA7, tsB4, tsB8, and tsB11, formerly called NTG 7, 4, 8, and 11, respectively (15, 20), were derived from WT virus after intracellular mutagenesis with nitrosoguanidine (18). Mutants 28 and 30 were derived from WT virus by extracellular mutagenesis with hydroxylamine by the method of Robb and Martin (14). Mutants of complementation group A do not replicate viral DNA (DNA-minus); group B mutants replicate viral DNA (DNA-plus) but either fail to produce V antigen and viral particles (tsB11) or make defective viral particles (tsB4, tsB8) as previously described (20). WT and mutant virus stocks were grown at 33 C in AH cells after inoculation of the monolayer cultures with virus aspirated from a well isolated plaque.

Productive infection of AGMK cells. AH monolayer cultures were inoculated with virus at input multiplicities of 10 plaque-forming units (PFU)/cell. After adsorption of the virus for 2 hr at room temperature, the cells were washed three times with Hanks salt solution (HSS) to remove unadsorbed virus, and the medium was replaced. For the assay of infectious virions, the cultures were harvested by freeze-thawing after 3 days of incubation at 39 or 41 C (restrictive temperatures) and after 5 days of incubation at 33 C (permissive temperature). Virions were plaque-assayed at 33 C as previously described (18). For the assay of intracellular infectious DNA, the infected cultures were harvested after 2 days of incubation at 39 or 41 C and after 4 days of incubation at 33 C. The infected monolayers were washed twice with tris(hydroxymethyl)-aminomethane (Tris)-buffered saline (TBS), and the intracellular viral DNA was selectively extracted by the method of Hirt (9). The Hirt supernatant fraction was dialyzed overnight at 4 C against TBS and assayed for the presence of infectious DNA by the method of McCutchen and Pagano (11).

Complementation. Complementation at 41 C was measured by inoculating mixedly infected tube cultures with 1 PFU of each of the two mutants to be tested per cell. Singly infected control cultures received the same multiplicity of each mutant. After adsorption, washing, and incubation at 41 C for 3 days, the samples

were freeze-thawed and titered at 33 and 41 C. Complementation indexes were measured as the ratio $(X + Y)_{33C} - (X + Y)_{39C}/(X)_{33C} + (Y)_{33C}$, where X and Y are yields of two mutant strains grown at 41 C and assayed at the temperatures indicated in the subscripts.

Conditions for radioactive labeling of cells. After incubation of infected cell cultures at 33 or 41 C for an appropriate period as indicated for each experiment, the medium in the 8-oz (45-cm² cell growing area) bottles used for monolayer cultures was replaced with medium containing 50 μCi of ³H-thymidine (New England Nuclear; 15 to 20 Ci/mmole) per ml prewarmed to 33 or 41 C. In experiments requiring a sudden temperature shift, the 8-oz bottles were almost completely submerged in a water bath at the appropriate temperature and were rapidly filled with HSS prewarmed to 33 or 41 C. The salt solution was then immediately aspirated by vacuum suction and replaced by the appropriate prewarmed medium for each experiment. This warming or cooling period required less than 1 min for completion. All pulse and pusle-chase incubations were carried out under water and were terminated by a rapid filling of the culture bottles with 4 C TBS prior to extraction of the DNA.

Extraction of DNA from infected cells. Viral DNA was selectively extracted from the infected cells as described by Hirt (9) and as summarized in the accompanying communication (19).

Preparation of marker DNA. SV40 DNA I and II labeled with ¹⁴C-thymidine were prepared as described in the accompanying communication (19).

Gel electrophoresis of viral DNA. The preparation of 1.5% agarose gels, the electrophoresis procedure, and the analysis of the gels were described in the accompanying paper (19). All gels were subjected to electrophoresis at room temperature for 2 hr at a potential of 10 V/gel.

Transformation of cells. Monolayer cultures of 3T3 cells were inoculated at an input multiplicity of 100 PFU/cell. After adsorption for 2 hr at room temperature, the cells were washed twice with TBS, suspended in 0.05% trypsin, diluted 10^{-2} in F12 medium containing 10% FCS, and plated in 75-cm² plastic flasks. Incubation was carried out at 33 C and at 38.5 to 39 C. After 1 day, the medium was replaced with the same medium containing 0.3% agar to prevent formation of secondary colonies. Transformed colonies were counted after 10 days at 38.5 to 39.0 C or after 20 days at 33 C. The medium was decanted, and the cells were washed, fixed with methanol, and stained with Giemsa solution. Colonial morphology was used to identify transformed clones.

RESULTS

Viral growth characteristics. Table 1 lists the yields of total infectious virions and of intracellular infectious DNA from a single cycle of growth at 33, 39, and 41 C after infection by intact virions. WT virus produced similar yields over the entire temperature range after the appropriate period of incubation required to complete a single cycle of replication in AH cells at each temperature (18). Mutant 4 synthesized infectious DNA at all temperatures but made 10^3 to 10^4 fewer virions at 39 and 41 C than at 33 C. Mutants 7, 28, and 30 were deficient in the production of both virions and infectious DNA at 41 C. The complete, or almost complete, restriction of infectious DNA production by mutants 28 and 30 at 41 C was significant, indicating that a host cell function could not substitute for the restricted viral function. The virion titers for mutants 28 and 30 at 41 C represented the background virus of the inoculum not completely removed by the washing of the cells after the initial 2-hr adsorption period.

TABLE 1. *Yield of virions and infectious DNA from one cycle of growth at permissive and restrictive temperatures after infection of AH cells by intact virions*[a]

Virus	Yield (PFU/ml)					
	Virion[b]			DNA[c]		
	33 C	39 C	41 C	33 C	39 C	41 C
WT	1×10^8	3×10^8	9×10^7	1×10^5	2×10^5	1×10^5
4	4×10^8	8×10^4	2×10^5	3×10^5	4×10^5	2×10^5
7	1×10^8	5×10^7	2×10^5	9×10^4	2×10^4	2×10^2
28	2×10^8	1×10^7	2×10^4	1×10^5	9×10^3	$<10^1$
30	2×10^8	2×10^7	1×10^4	2×10^5	1×10^4	$<10^1$

[a] Input multiplicity of 10 PFU/cell.

[b] Infected cultures were harvested after 3 days of incubation at 39 and 41 C and after 5 days of incubation at 33 C and were assayed for total virus production.

[c] Infected cultures were harvested after 2 days of incubation at 39 and 41 C and after 4 days of incubation at 33 C and were assayed for the production of intracellular infectious DNA.

Infectivity of mutant DNA. Infection of cells with extracted viral DNA of each of the DNA-minus mutants was found to be blocked at 41 C to the same extent as infection with intact virions (Table 2), indicating that the ts step remained defective subsequent to complete uncoating of the viral genome. The complete or almost complete restriction of infection by mutants 28 and 30 at 41 C was confirmed in this experiment by virion titration, a technique which should provide maximal sensitivity for the detection of viral replication.

Kinetics of expression of the ts functions in the viral growth cycle. Previous studies have shown that tsA7 and tsB4 could be classified as early or late mutants by the use of temperature-shift experiments (20). Similar experiments were performed to detect any possible variation in the kinetic characteristics of the three DNA-minus mutants, which might suggest that different functions were defective. Mutants 7, 28, and 30 could not be distinguished by temperature-shift

data (Fig. 1), but all were distinctly different from mutant 4. Since no significant difference in the heat lability of mutants 4, 7, 28, and 30 could be detected after 3 days at 41 C in F12 medium containing 10% FCS, the observed kinetic differences were not caused by inactivation of tsB4.

TABLE 2. *Yield of virions from one cycle of growth at permissive and restrictive temperatures after infection of AH cells by extracted viral DNA*[a]

Viral DNA[b]	Yield (PFU/ml)	
	33 C	41 C
WT	3×10^6	1×10^7
7	1×10^6	1×10^3
28	4×10^6	5
30	2×10^6	0

[a] Input multiplicity of 0.1 PFU/cell. Tube cultures were inoculated with viral DNA in the presence of 1 mg of diethylaminoethyl dextran per ml of TBS for 30 min, washed three times with TBS, and incubated in F12 medium with 10% FCS for 4 days at 33 C and 3 days at 41 C. The cultures were then frozen and thawed and plaque assayed for total virus production.

[b] Viral DNA was extracted from infected AH cells by the method of Hirt (9).

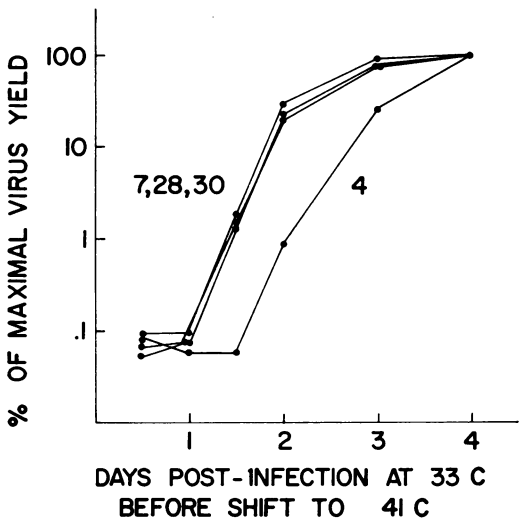

FIG. 1. *Virus production by mutant virus at the restrictive temperature after an initial incubation at the permissive temperature. A series of AH monolayer cultures infected at an input multiplicity of 10 PFU/cell were incubated at 33 C. At the indicated intervals, the cultures were shifted to 41 C. At 4 days after inoculation, all monolayers were frozen and thawed and titered for virus yield by plaque assay. The numbers in the figure designate the mutants tested.*

Heat inactivation. The thermal stability of the mutants in TBS at 50 C, *p*H 7, was tested to determine whether the defective gene products of each mutant were required to maintain the structural integrity of the virion particle. DNA-plus mutants 4, 8, and 11 were more heat labile than WT virus under these conditions, whereas DNA-minus mutants 7, 28, and 30 could not be distinguished from WT virus (Fig. 2).

Complementation studies. Table 3 presents the results of complementation tests. Each of the DNA-minus mutants clearly complemented DNA-plus mutant 11. No complementation be-

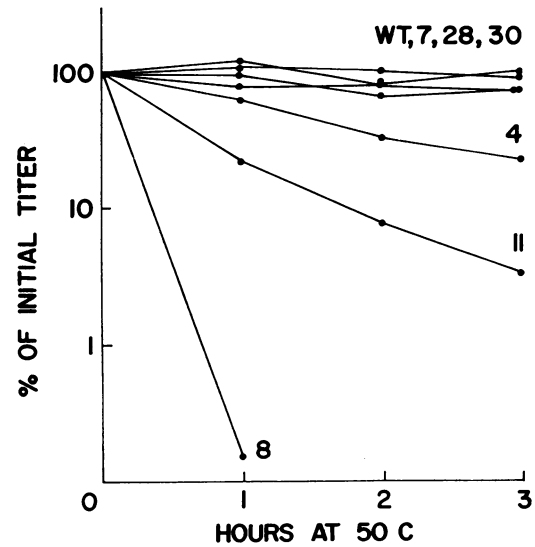

FIG. 2. *Heat stability of virion particles. WT and mutant virus in medium were diluted 10^{-2} in TBS (pH 7.0) prewarmed to 50 C. At intervals, samples were aspirated, rapidly diluted in 4 C TBS containing 2% FCS, and titered by plaque assay. The numbers in the figure designate which curves represent the data for each of the mutants.*

TABLE 3. *Complementation indexes between ts mutants at 41 C*[a]

Virus	Virus			
	11	7	28	30
11	1.2	123	88	162
7		0.8	1.4	1.0
28			1.5	0.6
30				1.1

[a] Complementation tests were performed as described in Materials and Methods. In all cases, the frequency of recombination and reversion was less than 1%.

tween mutants 7, 28, and 30 could be detected under the same experimental conditions, providing further evidence that the mutants were defective in the same function. These mutants have therefore been tentatively classified in complementation group A (15, 20). In mixed infection by tsA7 and tsB11, most of the progeny virus had the plaque characteristics of the DNA-minus parental virus (20), suggesting that the DNA molecule of the DNA-minus mutant could be replicated in the presence of a diffusible gene product produced by the DNA-plus parental virus. These results were expected, since tsA7 has previously been shown to be deficient not only in the production of viral DNA but also in the expression of certain late functions (20).

Synthesis of virus-sized DNA under permissive, semi-restrictive, and restrictive conditions. Viral DNA in infected monolayer cultures of AH cells was pulse-labeled with ^{3}H-thymidine, selectively extracted, and analyzed by gel electrophoresis to determine the relative quantities and kinds of viral DNA produced by WT and mutant virus under a variety of conditions (Table 4). The duration of the period of radioactive labeling of viral DNA was adjusted at each temperature so that approximately one-half of the newly replicated WT DNA would be in the completed DNA I configuration. At 41 C, mutants 7, 28, and 30 synthesized no detectable DNA although each replicated viral DNA indistinguishable from WT DNA at 33 C. At 39 C, each of the mutants produced viral DNA in significantly reduced quantities. Under these semi-restrictive conditions, no mutant replicative intermediate (RI) DNA or DNA II molecules accumulated in comparison to infection by WT virus. These findings suggested that, once a round of mutant DNA synthesis was initiated, replication continued without interruption.

TABLE 4. *The synthesis of viral DNA at 33, 39, and 41 C*[a]

Virus	^{3}H Counts/min				
	33 C		39 C		41 C
	Total	% DNA I[b]	Total	% DNA I	Total
WT	14,235	50	17,265	49	15,348
7	14,650	55	6,839	56	Background[c]
28	15,114	49	1,572	59	Background
30	13,103	54	720	62	Background

[a] AH cells were inoculated with WT and mutant virus at an input multiplicity of 10 PFU/cell and incubated at 33, 39, or 41 C for 72 hr. The infected cells were pulse-labeled with 50 μCi of ^{3}H-thymidine per ml for 30 min at 33 C and for 20 min at 39 and 41 C. The extracted DNA was analyzed by gel electrophoresis as described in Materials and Methods.

[b] Percentage of the total viral DNA synthesized during the labeling period that electrophoresed as DNA I.

[c] Background: no viral DNA peaks could be identified by gel electrophoresis.

Effects of temperature shifts on viral DNA synthesis. Since the character of the viral DNA synthesized during a period of a rapid decrease in the function of the ts gene product would provide the most unambiguous indication of the defective mutant function, temperature-shift experiments were performed to determine the quantitative and qualitative effects of a rapid change in temperature on the function of each of the early mutants. To label newly replicated viral DNA after a shift up in temperature, 10-min pulses with ^{3}H-thymidine were utilized at 41 C so that the duration of the labeling period would correspond to the approximate length of a single DNA replication cycle (19). Preliminary data (Table 5) showed that, 20 to 30 min after a sudden shift from 33 to 41 C, the new synthesis of viral DNA by mutants 7 and 28 was significantly decreased in comparison to WT virus. The relative proportion of DNA I to total viral DNA produced by the mutants at 41 C was similar to or slightly greater than that of the DNA produced in cells infected by the WT virus under the same conditions. Nevertheless, DNA synthesis continued well beyond the period of time required for a single cycle of viral replication. Mutant 30, however, exhibited little or no detectable function 20 to 30 min after a shift to 41 C.

TABLE 5. *The synthesis of viral DNA 20 to 30 min after a temperature shift from 33 to 41 C*[a]

Virus	^{3}H counts/min			
	33 C		41 C	
	Total	% DNA I[b]	Total	% DNA I
WT	13,054	53	10,744	3
7	13,396	47	4,734	6
28	12,866	52	1,402	11
30	13,288	54	Back-ground[c]	

[a] AH cells were inoculated with WT and mutant virus at an input multiplicity of 10 PFU/cell and were incubated at 33 C for 72 hr. The infected cells were pulse labeled with 50 μCi of ^{3}H-thymidine per ml for 30 min at 33 C or for 10 min after a 20-min temperature shift to 41 C. (Duration of labeling periods at 33 and 41 C was not comparable.) The extracted DNA was analyzed by gel electrophoresis as described in Materials and Methods.

[b] Percentage of total viral DNA synthesized during the labeling period that electrophoresed as DNA I.

[c] Background: no viral DNA peaks could be identified by gel electrophoresis.

The complete kinetics of the effects of both a shift up and a shift down in temperature on the function of tsA30 are shown in Fig. 3. Viral DNA synthesis in cells infected by mutant 30 terminated almost completely within a 10-min period after a shift from 33 to 41 C, whereas DNA production in cells infected by WT virus was stimulated under the same conditions. Thus, in infection by tsA30, the period of time required to nearly terminate viral DNA synthesis closely corresponded to the average length of a single replication cycle (19). A comparison of the gel patterns of the viral DNA produced by WT virus and mutant 30 during the period of rapidly declining DNA synthesis by the mutant (Fig. 4) indicated a distinct shift of mutant DNA to the late RI configuration (19) and to the final superhelical DNA I product in the first 10 min after a shift from 33 to 41 C. Little, if any, early RI (19) could be detected in cells infected by the mutant. The data taken together strongly suggested that the initiation of the replication process ceased at the time of, or shortly after, the temperature shift and that the RI forms which had already been initiated before the shift continued to be replicated and converted into DNA I.

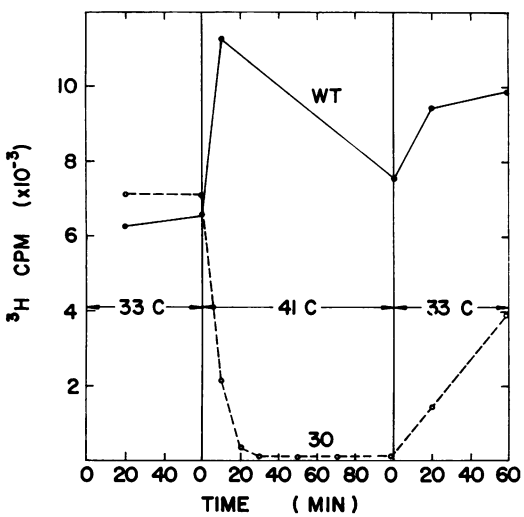

FIG. 3. *Effect of temperature shifts on the replication of viral DNA in AH cells infected by WT and tsA30 at an input multiplicity of 10 PFU/cell. The infected cells were first incubated for 72 hr at 33 C. Temperature shifts were performed as described in Materials and Methods. The cultures were labeled with 50 μCi of ^{3}H-thymidine per ml for 20 min at 33 C or 10 min at 41 C. Viral DNA was extracted by the Hirt technique and analyzed by gel electrophoresis. Total viral counts per minute are plotted above. Selected gel patterns of the viral DNA produced are shown in Fig. 4 and 5.*

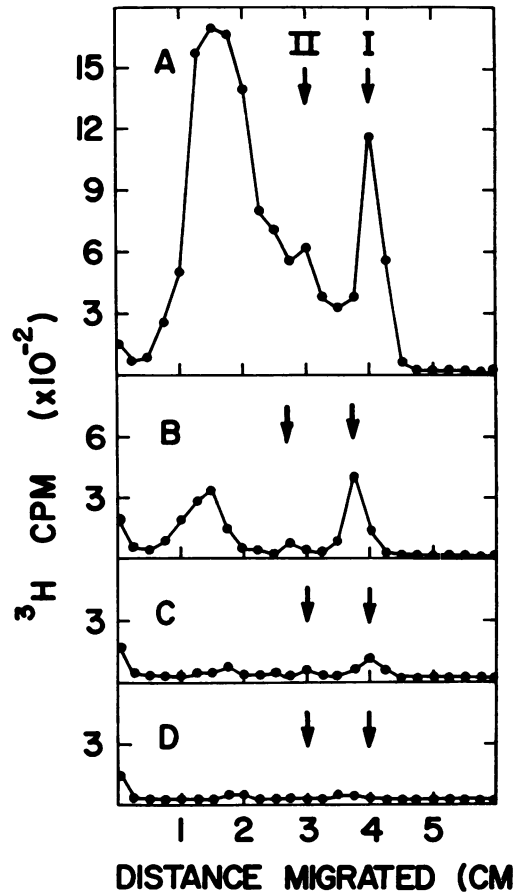

FIG. 4. *Gel patterns of WT and tsA30 DNA after a shift up in temperature from 33 to 41 C. The experimental conditions were described in Fig. 3. A, WT DNA labeled with ^{3}H-thymidine 0 to 10 min after a shift from 33 to 41 C. B, C, and D, Mutant DNA labeled with ^{3}H-thymidine 0 to 10, 10 to 20, and 20 to 30 min, respectively, after a shift from 33 to 41 C.*

The partial resumption of viral DNA production by tsA30 after a subsequent shift down in temperature required less than 20 min (Fig. 3), the approximate period of time required for a single cycle of synthesis at 33 C. The gel pattern of mutant DNA replicated after a sudden shift from 41 to 33 C (Fig. 5) indicated that both early and late RI, but not DNA I or II, had been produced in the first 20 min at 33 C. The quantity of mutant viral DNA synthesized early after a shift down in temperature varied in different experiments, but the gel pattern of the newly replictated viral DNA was consistently similar to that shown in Fig. 5B. The rapid effect of a temperature shift in either direction on DNA synthesis in cells infected by tsA30 provided evidence that the ts

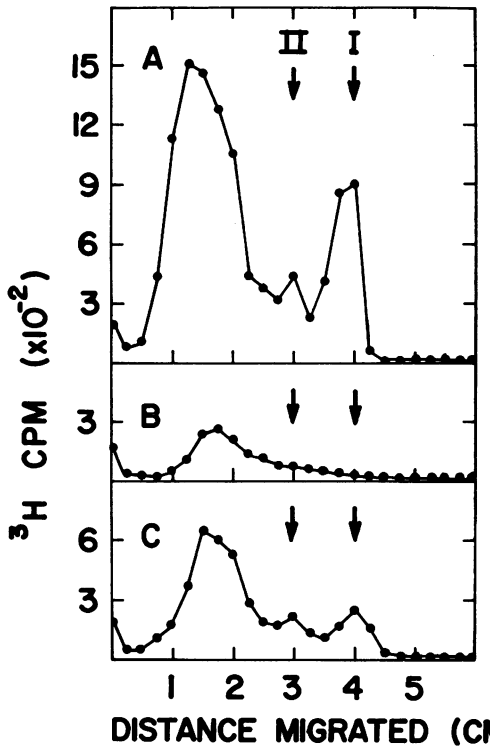

FIG. 5. *Gel patterns of WT and tsA30 DNA after a shift down in temperature from 41 to 33 C. The experimental conditions were described in Fig. 3. A, WT DNA labeled with ^{3}H-thymidine 0 to 20 min after a shift from 41 to 33 C. B and C, tsA30 DNA labeled with ^{3}H-thymidine 0 to 20 and 40 to 60 min after a shift from 41 to 33 C.*

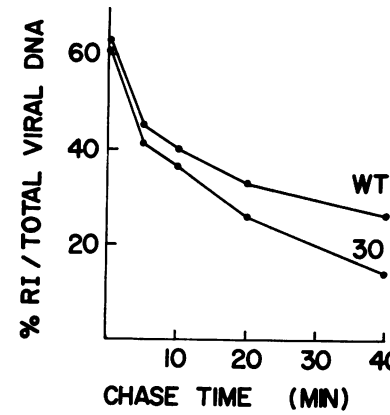

FIG. 6. *Processing of RI molecules in AH cells infected by WT virus and tsA30 at an input multiplicity of 10 PFU/cell. The infected AH cells were incubated at 33 C for 60 hr. Infected cultures were labeled with 25 μCi of ^{3}H-thymidine per ml for 20 min at 33 C. The bottles were aspirated, completely filled with 41 C HSS containing 2% FCS and an excess of unlabeled thymidine, and incubated underwater at 41 C for the periods of time shown above. Viral DNA was extracted by the Hirt procedure and analyzed by gel electrophoresis. The percent of total viral DNA in the RI form for each chase period is plotted above. The gel patterns of DNA extracted after a 40-min chase are shown in Fig. 7.*

gene product itself was directly required for the observed initiation function.

The data already presented have shown that RI molecules could be completed under restrictive conditions. Pulse-chase studies were also performed to compare more accurately the rates of viral DNA replication at 41 C in cells infected by WT and mutant virus. RI was prelabeled with short pulses of ^{3}H-thymidine at 33 C and chased with an excess of unlabeled thymidine at 41 C. RI molecules in cells infected by tsA30 were converted into DNA I at the same rate as in cells infected by WT during the first 5 to 10 min after the shift to 41 C (Fig. 6). Thereafter, RI in mutant-infected cells decreased somewhat more rapidly than in WT-infected cells. This apparent increase in the rate of conversion of RI to DNA I at 41 C in cells infected by tsA30 could be explained as a lack of reutilization of radiolabeled mutant DNA I as parental DNA in RI molecules as would be expected if the replication of new mutant RI molecules was no longer being initiated. Nevertheless, the chase was not 100% effective in cells infected by tsA30 at 41 C. This finding could not be explained on the basis of the available data. Possibly, the chase conditions resulted in a slight inhibition of DNA synthesis (13), even though the addition of deoxycytidine to the chase medium failed to increase the efficiency of the chase. However, the production of a few abnormal forms of viral DNA in cells infected by tsA30 under the experimental conditions could not be completely excluded. The gel patterns of the viral DNA synthesized in cells infected by WT virus and tsA30 at 33 C and chased with excess unlabeled thymidine for 40 min at 41 C are shown in Fig. 7. Similar studies, utilizing 15- and 40-min chases, showed that RI molecules initiated at 33 C in cells infected by mutants 7 and 28 were converted into DNA I at 41 C at a rate similar to the rate in cells infected by WT virus.

Thus, even though pulse-labeling experiments indicated that total mutant DNA synthesis was inhibited by a shift up in temperature, the rate of replication of RI molecules which had already been initiated was not found to be decreased in pulse-chase experiments. These findings provided strong evidence that the ts gene product of each of the group A mutants was required to initiate, but not to complete, a cycle of viral DNA replication. The period of time required to terminate

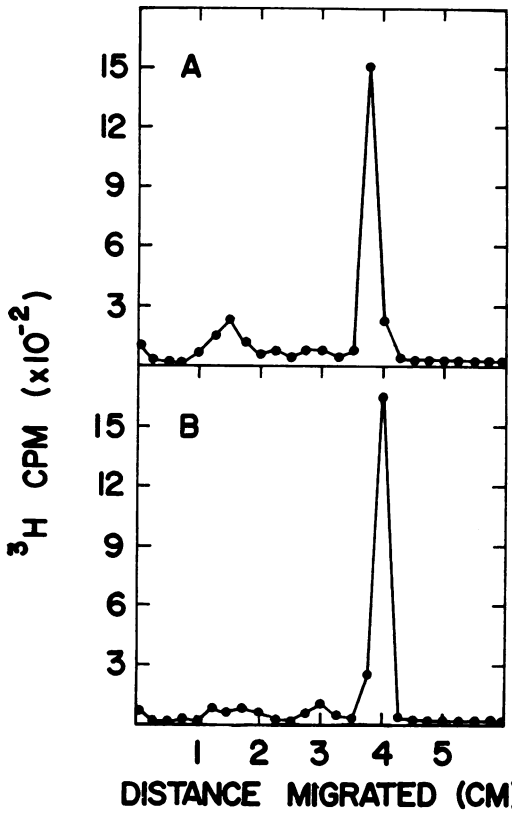

FIG. 7. *Gel patterns of WT and tsA30 DNA pulse-labeled with ^{3}H-thymidine at 33 C and chased with unlabeled thymidine for 40 min at 41 C as described in Fig. 6. A, WT virus; B, tsA30.*

viral DNA synthesis after a shift up in temperature varied considerably among the mutants. This difference probably reflected different degrees of leakiness of the same mutant function, depending on the nature of the mutant change in the ts gene product of each of the group A mutants.

Transformation. The transformation of 3T3 cells could not be accomplished at 41 C with either WT or mutant virus, since the cells survived very poorly at this temperature. Lower temperatures were therefore utilized. WT virus and mutant 4 transformed approximately the same number of 3T3 cells at 33 and 39 C, whereas early mutants 7, 28, and 30 transformed 25- to 155-fold fewer cells at 39 C than at 33 C (Table 6). The mutants were thus approximately 10-fold more deficient in the transformation of 3T3 cells at 39 C than in the replication of virus in AGMK cells at the same temperature, even though the input multiplicity of infection was 10-fold greater in the transforming infection. The difference could reflect either a difference in the quantitative

TABLE 6. *Transformation of 3T3 cells at 33 and 39 C*[a]

Virus	No. of transformed colonies[b]	
	33 C	39 C
WT	344	331
4	368	358
7	178	7
28	311	2
30	237	2
Control	0	0

[a] Transformation was performed as described in Materials and Methods.

[b] The number of transformed colonies at 33 C represented 2 to 6% of the total colonies plated as determined by further dilution and plating of the cells.

requirement for the same gene product in the transformation and in the replication processes, or more likely, a difference in the amount of gene product produced in the two different cell lines.

DISCUSSION

Mutants 7, 28, and 30 have been shown to belong to the same functional and the same complementation group. The ts gene product of these mutants is produced early in infection and is required to initiate, but not to complete, the replication of viral DNA. Since AGMK cells infected by two of the mutants produced little or no detectable infectious DNA at the restrictive temperature, the cell must be unable to supply the initiation function required for the replication of free viral DNA. Nevertheless, complementation studies suggest that a late mutant can produce a diffusible gene product capable of initiating the replication of the DNA of the early mutant, indicating that the mutant DNA molecule itself can be replicated at the restrictive temperature. The rapid effect of a temperature shift up or down on the ts function of mutant 30 suggests that the ts gene product is in fact the initiator molecule itself or an essential component of the initiator and not another molecule indirectly and remotely required for the production of the initiator. The possibility that the ts gene product is altered in such a way that it is unable to initiate binding to viral DNA but can replicate the same DNA after it has been irreversibly bound to it cannot be completely excluded. It seems unlikely, however, that the binding but not the replicating function of a molecule would be affected in all three of the mutants examined. The isolation of the viral initiator and the study of its precise molecular activity may provide important information on

the control of DNA synthesis in mammalian systems.

The evidence for a specific viral initiator provided here and the evidence for a specific initiation site on the viral DNA molecule presented by Nathans and Danna (12) indicate that SV40 has some genetic determinant(s) for a specific initiation system to control its own DNA replication and may be considered a replicon according to the theory of Jacob, Brenner, and Cuzin (10). Since the ts gene product of each of the three early mutants is also required to establish the stable transformation of restrictive cells, the viral replicon must play an essential role in the transformation process.

The evidence for the existence of the SV40 replicon suggests several possible models in which the DNA synthesis of contact-inhibited cells could be induced (4, 6, 7) under the partial control of the viral initiation system. Integration of viral DNA into the proper position in host DNA (3, 8, 16) could provide a new and uncontrolled initiation site for host cell initiators. Although host gene products apparently cannot initiate the replication of free, superhelical viral DNA, they may be able to interact with the viral initiation site in the integrated state. Alternatively the viral initiator could interact with a host DNA site. Both models imply an evolutionary similarity between viral and host initiation systems. A third alternative would require the interaction of the viral initiator with the integrated viral initiation site to stimulate cellular DNA synthesis. If the viral initiator is required to stimulate the DNA synthesis of transformed cells directly and continuously, then the ts function should be required not only to initiate but also to maintain the transformed state at the restrictive temperature.

In stable transformation, SV40 DNA has been shown to be integrated into cellular DNA by covalent bonds (3, 16). The exact role of this recombination of viral and cellular DNA in the transformation process remains to be determined. Integration may be only an indirect requirement for stable transformation by simply providing a mechanism for maintaining the continued presence of the viral genome and one or more of its gene products responsible for the continued stimulation of cell growth. On the other hand, it would be premature to exclude the possibility that the integration process is directly responsible in full or in part for the transformation of the host cell. Further studies of cells infected by each of the group A mutants may determine whether or not the mutant function is required to initiate and maintain the integration of the viral genome.

ACKNOWLEDGMENTS

This investigation was supported by special grant VC-98A for cancer research from the American Cancer Society, Ohio Division.

I am indebted to D. Anthony and L. Culp for their helpful discussions, and to Judith Kohout for her excellent technical assistance.

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