JOURNAL OF VIROLOGY, Aug. 1981, p. 481-489
0022-538X/81/080481-09$02.00/0

Vol. 39, No. 2

Replication of Simian Virus 40 DNA in Normal Human Fibroblasts and in Fibroblasts from Xeroderma Pigmentosum

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Simian virus 40 infection of semipermissive human diploid fibroblasts (HF), at early passage in cell culture, was compared with that of permissive established monkey cell lines. Viral DNA can be readily detected at 24 to 48 h postinfection at 37°C with a high multiplicity of infection, approaching 10% of that of monkey cells (TC7). The length of time necessary for replication of an average molecule of viral DNA was found to be indistinguishable in HF and TC7 cells. Strand elongation plus termination were assessed by following the accumulation of DNA I at 40°C from replicative intermediates of *tsA*30 prelabeled at 33°C, obviating isotope pool problems. Combined initiation and elongation of wild-type viral DNA was measured by density shift experiments involving a 5-bromodeoxyuridine chase of prelabeled [^{3}H]thymidine-labeled viral DNA. Determination of accumulation of viral T and V antigens supports the conclusion that the most likely basis for the reduced virus yield in HF cells results from the inefficiency of an early stage in virus infection, before or during uncoating. Similar results were obtained in fibroblasts derived from patients with xeroderma pigmentosum, suggesting that enzymes of UV repair are not required in unirradiated simian virus 40 DNA synthesis.

Papovaviruses, including simian virus 40 (SV40), initiate infection in a wide variety of cells from different species (for a review see reference 33). Typically, two types of infection may occur: permissive or nonpermissive. In the first case, full virus replication results in synthesis of progeny infectious viral particles. In the latter case, there is a partial expression of the viral genome, minimal or no viral DNA synthesis, and no progeny virus produced. This course may either be abortive or associated with persistence of the viral genome, typically in an integrated manner. In the case of SV40, permissive infection characteristically occurs in monkey cells and nonpermissive infection occurs in rodent cells. A third type of infection has been termed "semipermissive." In human cells, for example, SV40 infection results in appearance of virus-encoded early proteins (T antigens) and only limited progeny virus (2, 4, 9, 21, 22). There is little or no information on the rate and extent of viral DNA synthesis. We have undertaken a detailed study of viral DNA synthesis in early passage normal diploid human fibroblasts in an effort to understand better the basis for this semipermissivity; most notably, to determine whether such semipermissivity represents a quantitatively or qualitatively different virus-cell interaction than the typical permissive infection. Such a study takes on added interest at the present time for two reasons: first, there have been increasingly more investigations into the biochemistry of human papovaviruses and their relationship to SV40 (27, 33). Second, there are currently available several cell lines of fibroblasts derived from patients with hereditary disorders affecting cellular DNA repair and, possibly, DNA replication (11, 28). The latter represent a potential source of cell mutants to study the profound cellular contribution to viral DNA replication.

We have found that SV40 efficiently replicates its DNA in human fibroblasts. Moreover, the rate of viral DNA strand elongation, which is totally dependent on cellular functions, is indistinguishable from that of permissive monkey cells. Finally, we observed no difference among several complementation groups of fibroblasts derived from patients with xeroderma pigmentosum (XP), a disorder associated with a defect in repair of DNA damaged by UV light, indicating that such an enzyme(s) defective in this disorder is not required for replication of unirradiated viral DNA.

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MATERIALS AND METHODS

Virus. Wild-type SV40 (strain SV-S) and *tsA*30 were propagated at 37 and 33°C, respectively, at a low multiplicity of infection (MOI) in established green monkey kidney (GMK) cells as previously described (7, 19). Plaque assays were performed on TC7 or CV-1P monolayers.

Cell lines. Cell lines were propagated in the Dulbecco-Vogt modification of Eagle medium (DME) (Microbiological Associates) with 4.5 g of glucose per liter and 10% fetal calf serum (complete medium). GMK cell lines were passaged as previously described (13) and were obtained from the following sources: TC7 from J. Robb and J. Kaplan, respectively, CV-1P from D. Jackson, and Vero from the American Type Culture Collection (ATCC). Human fibroblast cultures were obtained at passage 3 to 6 from cell repositories and passaged a minimal number of times at 1:3 subcultures. HS lines were obtained from the Cell Culture Laboratory, University of California School of Public Health. Their properties are described by Smith et al. (30) and Zouzias et al. (34). CRL cell lines were obtained from the ATCC. The properties of those from XP patients are summarized by Robbins et al. (23). The familial relationships of patient 9 (XP9BE) are reported by Lynch et al. (16). All cell lines were verified to be free of mycoplasma by cultivation procedures, except for CRL 1199 and CRL 1200 which were positive as reported by the ATCC. The tissue origins and XP phenotypes are summarized in Table 1.

Chemicals and solutions. 5-Bromodeoxyuridine (BUdR) and deoxycytidine (CdR) were obtained from Sigma Chemical Co. (A grade). Fluorodeoxyuridine (FUdR), thymidine (TdR), and cytosine-1-β-D-arabinofuranoside-HCl (araC) were obtained from Calbiochem. Solutions were prepared in distilled water and filter sterilized.

Viral DNA synthesis. Subconfluent or confluent cultures in 60-mm petri dishes were infected with 0.5 ml of an unpurified virus preparation as previously described (20). At appropriate times postinfection (p.i.), cultures were radiolabeled for 1- to 4-h periods with [^{3}H]TdR at 10 μCi/ml (specific activity, 50 Ci/mmol, Schwarz/Mann). Viral DNA was extracted by the Hirt procedure (12) and quantitated as forms I and II (18 to 21S) by sedimentation on neutral sucrose gradients (NSG), using purified [^{14}C]DNA I as an internal marker as described previously (29). Incorporation was determined after precipitation with 5 to 10% trichloroacetic acid.

Viral DNA strand elongation. Confluent cultures in T25 flasks were infected with 0.2 ml of virus as described above. After 3 to 7 days at 33°C (depending on the cell line and virus pool), triplicate cultures were radiolabeled with 20 μCi of [^{3}H]TdR per ml for 20 min (TC7 cells) or 30 min (human fibroblasts) at 33°C and then shifted rapidly to a water bath equilibrated at 40.5°C. Incubations were terminated at the time of shift to 40.5°C and at 10-min intervals by plunging the flask into ice, rapid washing (twice) by cold phosphate-buffered saline, and Hirt extraction. The pooled Hirt supernatant was layered onto NSG, and viral DNA I was determined. In those experiments involving infection of CRL 1161 and CRL 1166 by *tsA*30, the region of the NSG containing DNA I was identified on the basis of the [^{14}C]DNA. The peak fractions were pooled, dialyzed against 0.15 M NaCl-0.01 M EDTA-0.01 M Tris-hydrochloride (pH 7.6), and centrifuged to equilibrium in CsCl (ρ = 1.56 g/cm) containing 100 μg of ethidium bromide per ml. Quantitation of [^{3}H]DNA was corrected for recovery of admixed [^{14}C]DNA I in this case.

Kinetics of viral DNA reentry. The procedure used was a modification of that employed by Roman and Dulbecco (24, 25) for polyoma virus-infected mouse cells. Confluent cells were infected in 60-mm dishes with wild-type SV40. At 24 or 40 h p.i. [^{3}H]TdR was added at 10 μCi/ml for 60 min, followed by 10^{-5} M TdR for an additional 60 min. The medium was then removed, the culture was washed once in DME, and BUdR medium was added (5×10^{-5} M BUdR, 2×10^{-5} M FUdR, 1×10^{-5} M deoxycytidine [CdR] in complete medium). Viral DNA was extracted after various durations of chase in BUdR by the Hirt procedure. DNA I was isolated from pooled triplicate cultures after sedimentation on alkaline sucrose gradients. After neutralization, HL (DNA substituted with BUdR in one strand) and LL (no BUdR substitution) molecules were separated by equilibrium centrifugation in CsCl, ρ = 1.72 g/cm. The percentage of ^{3}H cpm found in HL DNA [HL/(HL + LL) × 100] was determined. Incorporation of [^{3}H]CdR (1 μCi/ml in 10^{-5} M) into DNA I was linear in infected TC7 or HS27 cells over a 6-h period in BUdR medium in control experiments; the rate of incorporation of BUdR into either viral or cellular DNA was approximately 60 to 70% of that observed for TdR in comparable medium.

Virus production in human fibroblasts. Confluent 60-mm petri dishes were infected with 0.2 ml of virus. After adsorption for 2 h, the inoculum was removed, the monolayers were washed five times with DME, 5 ml of complete medium was added, and the

TABLE 1. *Human fibroblastic cell lines*[a]

Cell line	XP designation (genetic group)	Tissue of origin and age of patient
HS 27	None (normal)	Foreskin, newborn
HS 74	None (normal)	Bone marrow, fetal
CRL 1161	XP9BE (C)	Skin, 12 yr
CRL 1162	XP4BE (variant)	Skin, 27 yr
CRL 1165	None (parent of XP9BE)	Skin, 54 yr
CRL 1166	XP2BE (C; sibling of XP9BE)	Skin, 22 yr
CRL 1167	None (parent of XP9BE)	Skin, 54 yr
CRL 1170	XP1BE (C)	Skin, 27 yr
CRL 1199	XP11BE (B)	Skin, 28 yr
CRL 1200	XP7BE (D)	Skin, 11 yr
CRL 1204	XP10BE (C)	Skin, 16 yr
CRL 1223	XP12BE (A)	Skin, 7 yr

[a] Origins and properties of cell lines are described in the text.

cultures were harvested by freezing and thawing at appropriate times.

Serological assays. Microcomplement fixation for SV40-specific antigens were performed as described previously (20). T antigen(s) was assayed with a hamster antiserum to a transplantable SV40 tumor. V antigen was assayed with a hyperimmune monkey antiserum prepared against viral capsids, which was nonreactive with T^+ V^- cells. Infected extracts were prepared from duplicate or triplicate 100-mm petri dishes infected with 1 ml of wild-type virus, undiluted or after dilution in DME containing 1% fetal calf serum.

RESULTS

Viral DNA synthesis in human fibroblasts. When subconfluent or confluent cultures of human fibroblasts are infected with SV40 at multiplicities of infection (MOIs) of greater than 10 at 37°C, minimal cytopathic effects are observed. Virus yield (in PFU) has been reported to vary over a wide range from minimally above the absorbed level to 1 to 3 PFU per cell (4, 22) in contrast to greater than 100 PFU per cell (10^9 to 10^{10} per culture) commonly observed for permissive monkey cell lines (33). A number of early passage human fibroblast cell strains were tested for viral DNA synthesis at 2 days postinfection with SV40. The results are summarized in Table 2. Different cell strains vary in their susceptibilities, but readily detectible levels of viral DNA are observed in the case of fibroblasts from both presumably normal donors and those from patients with the hereditary disorder XP. Table 3 shows a comparison between the human fibroblasts HS27 and three permissive monkey cell lines. Due to the multiple variables among the different cultures, these results can only be considered approximations. Nonetheless, it is clear that considerable viral DNA synthesis can occur in human fibroblasts at a level approaching 10% of that of permissive cells. A more detailed comparison between HS27 and TC7 cells was undertaken in an effort to understand the basis for this apparent reduced rate of viral DNA synthesis in human fibroblasts.

TABLE 2. *Synthesis of SV40 DNA in human fibroblasts*[a]

Cell line[b]	Viral DNA[c]	
	cpm per culture	% of total counts
HS 27	40,560	9.4
HS 74	≤3,150[d]	≤1.4
CRL 1161	10,900	4.3
CRL 1162	7,920	2.0
CRL 1199	31,700	6.8
CRL 1200	39,600	7.7
CRL 1223	56,700	7.7

[a] Confluent monolayers in 60-mm petri dishes were infected with wild-type SV40 at an MOI of 20 to 40 PFU per cell and labeled for 2 h with $[^3H]$TdR at 48 h p.i. as described in the text.

[b] Cell lines are listed in Table 1.

[c] Viral DNA was quantitated by NSG analysis of the supernatant fraction by the Hirt procedure as described in the text. Extracts from uninfected cells processed by the same method averaged approximately 2,400 cpm and 1.5% of the total counts.

[d] No distinct peak was observed on NSG.

TABLE 3. *Synthesis of SV40 DNA in monkey cells*[a]

Cell line	Viral DNA[b] at:			
	24 h p.i.		48 h p.i.	
	cpm per culture	% of total counts	cpm per culture	% of total counts
T C7	3.8×10^5	24	2.6×10^6	75
CV-1P	3.5×10^5	34	$>1.3 \times 10^5$[c]	43
Vero	6.4×10^4	9.3	1.7×10^5	37
HS27	1.0×10^4	1.9	4.1×10^4	9.4

[a] Confluent monolayers were infected as described in Table 2 except for Vero cells in which the MOI was approximately 5 PFU per cell.

[b] Viral DNA was determined as in Table 2.

[c] Underestimate of viral DNA due to evident viral cytopathicity.

DNA strand elongation was determined p.i. with wild-type SV40 or a temperature-sensitive mutant (*tsA*30) of SV40 defective in initiation of viral DNA synthesis at the restrictive temperature. Cultures were infected at 33°C and incubated until viral DNA synthesis was readily measurable. Tritiated TdR was added for 20 to 30 min to permit labeling of the intracellular nucleotide and replicating viral DNA pools. Cultures were then shifted to 40°C, and radioactivity in progeny viral DNA I was determined immediately and at 10-min intervals. We would expect accumulation of radioactivity in DNA I to be a direct reflection of strand elongation since it had previously been reported that initiation of new rounds of viral DNA synthesis in monkey cells is inhibited within 10 min for this mutant. Figure 1A shows the results for TC7, and Fig. 1B shows the results for HS27. In cells infected with *tsA*30, radioactivity in DNA I increases in monkey cells for approximately 20 min as expected, which is consistent with the inactivation of A function (~10 min) and the time necessary to complete an already initiation-replicative intermediate (~15 min) (15, 30). A similar result was obtained for HS27 cells infected with *tsA*30. The marked decrease in accumulation of DNA I after 20 min at 40°C cannot be attributed to toxicity associated with

the virus infection or radiolabeling procedure or both since the parallel cultures infected with wild-type SV40 show progressive accumulation of DNA I for at least 60 min after a shift to 40°C. Direct comparison of the level of viral DNA synthesis between the two cell lines is not possible in view of the different conditions of infection, etc. (see figure legends).

We next investigated the efficiency of reentry of viral DNA in the two cell lines by the method of Roman and Dulbecco (25). Cells were infected with wild-type SV40, and the replicating DNA pool was labeled with [^{3}H]TdR. Subsequent replication was determined by incorporation of BUdR. The proportion of DNA I of intermediate density was determined at 24 and 40 h p.i. in monkey cells and 40 h p.i. in human cells since there was insufficient DNA replication at the earlier time p.i. to obtain accurate determinations. As previously observed for polyoma virus-infected mouse cells and SV40-infected monkey cells, using a somewhat different labeling regimen, reentry of viral DNA molecules in permissive monkey cells is incomplete, with a plateau value of less than 100% (10, 24, 25). Although the plateau value can vary considerably with the time p.i., the period during which reentry is observed is reasonably constant, being approximately 4 h after addition of BUdR (Fig. 2). The results with the infected human fibroblasts are similar (e.g., within experimental error considering the number of manipulations involved),

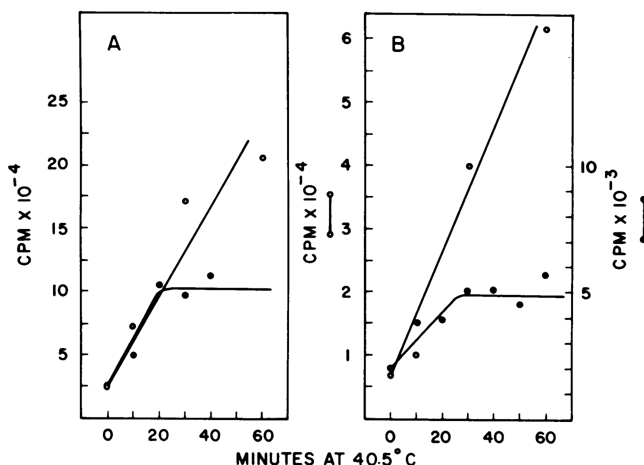

FIG. 1. *Viral DNA strand elongation. Confluent cultures were infected by tsA30 (●) or wild-type SV40 (○) at 33°C, pulse-labeled with [^{3}H]TdR, and shifted to 40.5°C in the continuous presence of isotope for quantitation of accumulation of viral DNA I on NSG as described in the text. (A) Monkey cells (TC7) infected for 3 days; (B) human cells (HS27) infected for 4 days. The MOI was comparable for both cell lines (20 to 40 for wild type and 10 to 20 for tsA30).*

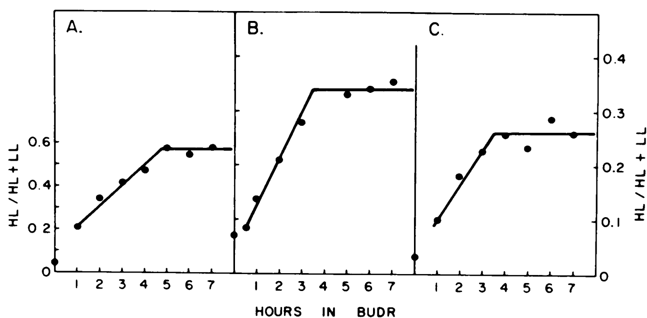

FIG. 2. *Kinetics of reentry of viral DNA synthesis. Confluent cultures infected with wild-type SV40 were pulse-labeled with [^{3}H]TdR, followed by a chase in BUdR medium as described in the text. The proportion of viral [^{3}H]DNA I converted to intermediate HL density was calculated after purification by Hirt fractionation and alkaline sucrose gradient and cesium chloride-ethidium bromide equilibrium sedimentations. (A) Monkey cells (TC7) at 24 h p.i.; (B) monkey cells (TC7) at 40 h p.i.; (C) human cells (HS27) at 40 h p.i.*

particularly when compared with those of monkey cells infected for the same length of time (compare Fig. 2B and C). This procedure measures all stages of viral DNA replication. Inasmuch as we have shown in the preceding section that those steps after initiation do not differ in the two infected cell lines, we can conclude that no major difference exists in reinitiation of viral DNA synthesis as well.

Accumulation of viral antigens in infected cells. These results on the similarity in the rates of viral DNA synthesis between semipermissive human cells and permissive monkey cells suggest that the reduced level of viral DNA in the former is a reflection of a defect in a step earlier in infection. Consistent with this model, other investigators have reported that not all human cells accumulate detectable T antigen by fluorescent antibody p.i. by a moderately high MOI (i.e., sufficient to induce T antigen in greater than 90% of monkey cells) (2, 3, 21). We similarly observed that accumulation of T antigen by complement fixation at 24 h p.i. is reduced in HS27 cells as compared with TC7 cells when the same inoculum is used to infect parallel cultures (Table 4). Cultures were harvested at 24 h p.i., and an inhibitor of DNA synthesis (araC, 1×10^{-5} M) was included in the medium to minimize differences secondary to the different levels of DNA synthesis in the two cell lines. (No difference was observed in preliminary experiments with and without araC.) However, as the virus input is increased from 10 to 20 to 50 to 100 PFU per cell, there is only a slight increment in T antigen in monkey cells, whereas T antigen continues to increase proportionately in HS27 cells. At low MOI, there is also a marked difference in T antigen accumulation. The effect of this difference between the two populations is further indicated by the results in Table 5 in which accumulation of virus particles as V antigen was determined by complement fixation at a high MOI. There is a fivefold-lower level in HS27 cells similar to the reduction in the level of viral DNA. Fluorescent-antibody studies for viral capsid proteins confirm that a minority of human cells are infected at all time points in contrast to >90% of the monkey cells (data not shown). There is also a comparable reduced yield of virus at different times p.i. (Table 6). A human fibroblast (HS74) cell line which showed minimal viral DNA synthesis in Table 2 has a still further reduced virus yield.

Viral DNA synthesis in human fibroblasts from XP. The studies described above indicated that the semipermissive nature of infection of early passage human fibroblasts with SV40 is consistent with a readily detectable level of viral DNA synthesis in those cells which are efficiently infected. Therefore, we sought to determine whether viral DNA replication occurred efficiently in cells derived from patients with hereditary defects in DNA synthesis or repair. We chose to examine fibroblasts from patients with XP, a disease characterized by skin tumors and defects in repair of damage to DNA by UV light. Multiple complementation groups have been reported, some of which might logically be expected to be defective in functions associated with synthesis of unirradiated DNA (one or

TABLE 4. *Accumulation of T antigen by complement fixation*[a]

Virus input (PFU × 10^{-6})	T-antigen titer for:	
	TC 7	HS27
200	640	170
40	160–480	20–43
13	80–160	10–13
7	80	<3

[a] Confluent 100-mm petri dishes were infected as described for microcomplement fixation in the text. A single stock of wild-type virus was diluted in medium with 1% serum for parallel infections. Two dishes of infected TC7 cells were extracted in 0.5 ml, and three dishes of infected HS27 cells were extracted in 0.25 ml for antigen titration. Data are presented as antigen per infected petri dish. There were similar cell numbers in cultures of both cell lines.

TABLE 5. *Accumulation of T and V antigens by complement fixation*[a]

Time p.i. (h)	TC7		HS 27	
	T antigen	V antigen	T antigen	V antigen
24	320	640	128	128
48	640	6400	256	1280
72	320	12,800	256	640

[a] Microcomplement fixation of infected cell extracts was performed as described in Table 4. 2×10^8 PFU of SV40 were used for infection in all cases.

TABLE 6. *Virus yield in human fibroblasts*[a]

Time p.i. (h)	PFU per ml in:		
	CV-1P	HS27	HS74
2	$<1.0 \times 10^{6}$[b]	$<1.0 \times 10^{5}$[b]	3.0×10^5
24	1.0×10^6	1.0×10^5	1.0×10^5
48	6.4×10^7	3.6×10^6	1.0×10^5
72	1.1×10^8	6.2×10^6	3.8×10^6
120	NT[c]	1.2×10^7	3.4×10^6
168	NT	1.5×10^7	3.8×10^6

[a] Infected cultures were harvested for virus as described in the text and assayed for plaque formation on CV-1P monolayers.

[b] No plaque observed at lowest dilution tested.

[c] NT, Not tested.

more polymerases, ligase, etc.) as well as those steps unique to UV-damaged DNA, such as pyrimidine dimer excision. As shown in Table 2, representatives of three of five complementation groups synthesize viral DNA comparable to HS27 (CRL 1223, CRL 1199, CRL 1200) at 48 h p.i. and earlier times (data not shown). CRL 1162 had a reduced level; however, incorporation into viral DNA was consistent with several other cell lines in the same experiment and appears to reflect experimental conditions rather than an intrinsic defect in the cell line (see Table 7 for comparison). CRL 1161, on the other hand, consistently showed a reduced incorporation relative to other cell lines tested in the same experiment under different conditions of infection (e.g., earlier times p.i. and when confluent cultures were infected and maintained in medium putatively depleted for growth factors). Further analysis of CRL 1161 and genetically related cell lines indicated that the defect was most likely intrinsic to the cell line and genetic in nature but probably unrelated to the XP phenotype or a defect in DNA strand elongation. In Table 7, viral DNA synthesis in CRL 1161 is compared with that of a number of additional phenotypically or genotypically related cell lines. Whereas CRL 1161 and CRL 1166, derived from an affected (XP) sibling, show diminished viral DNA synthesis, two other representatives of the same complementation group (CRL 1170, CRL 1204) have considerably higher levels. Both parents (CRL 1165 and CRL 1167) also show higher levels. DNA strand elongation was determined

TABLE 7. *Synthesis of SV40 DNA in human fibroblasts related to CRL 1161*[a]

Cell line[b]	cpm of DNA per culture[c]	
	Infected	Infected-uninfected[d]
CRL 1161	6,420	2,910
CRL 1165	8,720	6,500
CRL 1166	3,055	1,720
CRL 1167	6,945	4,145
CRL 1170	12,270	11,050
CRL 1204	11,235	9,865

[a] Confluent monolayers were infected or the medium was changed without infection (uninfected) as described in Table 2.

[b] Cell lines and their genetic relatedness are as listed in Table 1.

[c] Viral DNA in the Hirt supernatant was quantitated as described in Table 2.

[d] Viral DNA was corrected by subtraction of comparable NSG fractions obtained from parallel extracts from uninfected cells. Trichloroacetic acid-precipitated counts per minute in the total cell extract averaged 2.9×10^5 for infected cultures and 2.5×10^5 for uninfected cultures.

in CRL 1161 and CRL 1166 p.i. with *tsA*30 at 33°C and shift to 40°C (Fig. 3). The results are similar to those in Fig. 1 for monkey cells and HS27. It should be noted that because of the low level of viral DNA being measured, it was necessary to purify further the appropriate fractions containing DNA I to free it of contaminating cellular DNA. Therefore, the data have been corrected for recovery of added [^{14}C]DNA I. Variability was also observed among the several experiments performed. It was not possible to determine rates of reentry of viral DNA due to insufficient incorporation. The results suggest that CRL 1161 and CRL 1166 are likely to be examples of a reduced efficiency of infection related to virus penetration or uncoating or both as previously described (1). Consistent with this interpretation, we have observed reduced accumulation of T antigen by complement fixation.

DISCUSSION

SV40 infection of human fibroblasts has been found to result in readily detectable replication of viral DNA and production of infectious progeny. We determined the rate of viral DNA strand elongation directly and initiation indirectly through kinetics of reentry in a normal fibroblast and found no significant difference from similarly infected permissive monkey cells. Comparable strand elongation rates were also observed in two cell strains derived from patients with XP. Similar results were obtained under conditions of varied effective MOIs (compare Fig. 1B, and 3A and C). These measurements should best be considered approximations. Our analysis of strand elongation assumes that the period being measured is predominantly a reflection of events throughout strand elongation rather than those at a single step. The distribution of viral replicative intermediates has been reported as non-

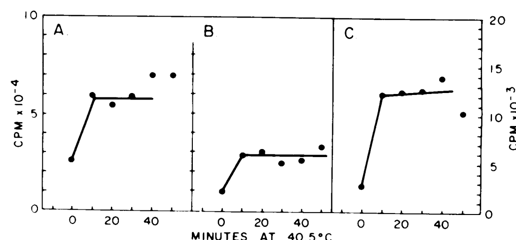

FIG. 3. *Viral DNA strand elongation in XP fibroblasts. Cultures were infected with tsA30 at 33°C and analyzed as in Fig. 1. Viral DNA I was quantitated after Hirt extraction and NSG and cesium chloride-ethidium bromide equilibrium sedimentations. (A) CRL 1161 cell infected for 4 days at an MOI of 20 to 40; (B) CRL 1166 cell infected for 4 days at an MOI of 20 to 40; (C) CRL 1161 cell infected for 7 days at an MOI of 1 to 5.*

random with accumulate of late replicative intermediates in SV40-infected monkey cells and it is unclear to what extent that phenomenon would distort the expected results (26, 31). It should also be stressed that all analyses on DNA synthesis disproportionately involve those infected fibroblasts which most efficiently replicate viral DNA since the contribution of others in the incorporation of radioactive precursors would logically be insignificant by comparison. Despite these limitations, the conclusion appears warranted that at least a subpopulation of cells in early passage line of human fibroblast can perform the steps of SV40 DNA replication as efficiently as monkey cells. This would imply that the cell factors responsible for permissivity that operate at the level of DNA initiation and elongation are equivalent. Previous studies on papovavirus DNA replication in vitro had shown that nonpermissive cells could provide one or more factors (e.g., cytosol fraction components) which facilitated DNA strand elongation in nuclear or subnuclear systems (6, 8). It has not been possible thus far to measure initiation of viral replicons in a cell-free system.

The basis for the semipermissivity of human fibroblasts appears to reside predominantly at an earlier stage of infection. It has been previously reported that SV40 infection of human cells was less efficient than that of monkey cells, as determined by accumulation of T antigen in the nucleus (2-4, 21). Cell lines have been identified with varied susceptibility for virus-mediated transformation and accumulation of T antigen by immunofluorescence assay (designated as susceptible, normal, and resistant) (2). However, in all cases, there was a relatively low percentage of positive cells.

Even at a high MOI when the number of T antigen-positive cells reached maximal values, less than 30% of the most highly susceptible cell strain culture is T antigen positive. Others are considerably reduced and have not yet shown evidence of plateau values (0.2 to 4%). The slopes of curves for the percentage of T antigen-positive cells as a function of input of virus are consistent with a single-hit event (2). Our data on accumulation of T antigen(s) at 24 h p.i. by complement fixation show a similar phenomenon. T antigen levels are reduced as compared with 6 monkey cells at an equally low to moderate MOI and approach the latter at a high MOI, due to already maximal levels in monkey cells at a lower MOI. We would assume that the human fibroblast HS27 in our study is in the normal or, more likely, susceptible category of Aaronson and Todaro (2), whereas CRL 1161 cells are in the resistant class. It appears that two phenomena are operative: a reduced efficiency of T antigen expression and a mixed cell population, although the less likely possibility that the latter could represent an extreme case of the former has not been ruled out.

The basis for reduced T antigen levels appears to be a reflection of inefficient steps before or during uncoating of virus. Aaronson (1) found that T antigen induction p.i. with viral DNA (with DEAE-dextran as facilitator) was as efficient in normal and susceptible fibroblasts. HS74, which shows reduced viral DNA synthesis or infectious virus as compared with HS27, upon virion infection in this study is capable of highly efficient viral DNA replication and T antigen synthesis from intracellular SV40 DNA as we have previously demonstrated in a clone transformed by a fragment of the SV40 genome bearing the early region and the origin of DNA replication (33). It should be noted, however, that Carp and Sokol (5) saw no relative improvement in the efficiency of T antigen induction with viral DNA in a comparison between primary African GMK cells and the human fibroblast cell line W1-38; GMK was approximately 50-fold better with virion or DNA.

Therefore, it would appear most likely that semipermissive infection of human fibroblasts with virions containing nondefective viral genomes is predominantly the summation of normal infection in a minority of the cells. Even these cells have a lower efficiency of infection, such that the effective MOI being employed is moderately or markedly reduced as compared with that of GMK cells which were used to titrate the virus preparation. Viral DNA replication and subsequent events appear to follow in a fashion consistent with those of permissive cells. Viral capsids (as V antigen assayed by complement fixation) and infectious virions accumulate at a level roughly proportional to the level of viral DNA synthesis at high input MOI as shown in this study. Immunofluorescence assays for T and V antigen have previously shown concordance between the number of antigen-positive cells in some (2) but not all studies (4). In our experiments and the study described in reference 2, conditions were used which have been shown to minimize accumulation of viral particles with defective genomes (wild-type strain SV-S at low MOI).

As an initial effort toward the possible exploitation of hereditary human disorders to identify host function involved in SV40-cell interaction, we investigated the efficiency of viral DNA synthesis in human fibroblasts derived from XP. Representatives of multiple complementation groups involved in excision repair and a "var-

iant" class presumably defective in postreplication repair were included (29). The enzymatic bases for these defects in repair of UV damage have not been unambiguously determined. Current models of repair based on procaryotic systems invoke in large part the involvement of multiple enzymatic functions which could be relevant in some cases to semiconservative viral DNA replication as well. The data that we obtained fail to demonstrate any deficiency in viral DNA synthesis. In the two related cell strains which showed reduced viral synthesis upon preliminary testing (CRL 1161 and CRL 1166), DNA strand elongation was not abnormal in contrast to the prediction if the putative enzymatic defect in the XP phenotype were responsible. Two incidental positive findings are that early passage fibroblasts from adult donors (CRL) support viral DNA synthesis as well as do HS27 cells derived from a newborn and that accurate DNA strand elongation measurements can be performed even under rather poor efficiency of infection as in CRL 1161, emphasizing the possible utility of the approaches to other poorly permissive virus-cell systems.

It should be noted that this model for the basis of semipermissive infection in human cells need not apply to other semipermissive SV40-cell interactions; most notably, with rodent cells as those of the hamster (14, 22). Alternatively, SV40 infection of human cells has been reported in which extensive viral cytopathic effects are observed (17). That SV40-cell interaction also has the unusual property of a very high proportion of defective viral genomes, even after infection at low MOI (18).

ACKNOWLEDGMENTS

We thank our colleagues for providing cell lines and the ATCC Cell Repository and the Cell Culture Laboratory at the Naval Biochemical Research Laboratory, Oakland, Calif., under contract E-73-2001-NO1 within the Special Virus-Cancer Program, National Institutes of Health. This investigation was supported by Public Health Service research grant CA-23002 from the National Cancer Institute to H.L.O. M.L.S. was supported by a Public Health Service research fellowship from the National Cancer Institute.

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