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Stimulation of Host Centriolar Antigen in TC7 Cells by Simian Virus 40: Requirement for RNA and Protein Syntheses and an Intact Simian Virus 40 Small-t Gene Function

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Simian virus 40 (SV40) stimulated a host cell antigen in the centriolar region after infection of African green monkey kidney (AGMK) cells. The addition of puromycin and actinomycin D to cells infected with SV40 within 5 h after infection inhibited the stimulation of the host cell antigen, indicating that de novo protein and RNA syntheses that occurred within the first 5 h after infection were essential for the stimulation. Early viable deletion mutants of SV40 with deletions mapping between 0.54 and 0.59 map units on the SV40 genome, *dl*2000, *dl*2001, *dl*2003, *dl*2004, *dl*2005, *dl*2006, and *dl*2007, did not stimulate the centriolar antigen above the level of uninfected cells. This indicated that an intact, functional small-t protein was essential for the SV40-mediated stimulation of the host cell antigen. Our studies, using cells infected with nondefective adenovirus-SV40 hybrid viruses that lack the small-t gene region of SV40 ($Ad2^+ND1$, $Ad2^+ND2$, $Ad2^+ND3$, $Ad2^+ND4$, and $Ad2^+ND5$), revealed that the lack of small-t gene function of SV40 could be complemented by a gene function of the adenovirus-SV40 hybrid viruses for the centriolar antigen stimulation. Thus, adenovirus 2 has a gene(s) that is analogous to the small-t gene of SV40 for the stimulation of the host cell antigen in AGMK cells.

Simian virus 40 (SV40), an oncogenic DNA-containing virus, multiplies productively in African green monkey kidney (AGMK) cells. After SV40 infection of permissive cells, the early genes of SV40 are transcribed and translated before the viral DNA synthesis begins (45, 46). After the onset of viral DNA replication, late gene transcription is initiated (19, 21, 22, 34), followed by translation of late mRNAs. Early genes continue to be expressed after the initiation of DNA synthesis (4, 48, 56). In nonpermissive cells, SV40 DNA may become integrated into the host DNA (10, 14, 49, 55, 57). However, neither viral DNA replication nor transcription of SV40 late genes occurs in nonpermissive cells (18, 23, 40). It has been shown that the early region of SV40 codes for at least two polypeptides, the large-T antigen and the small-t antigen (5, 42, 53). Large-T antigen has been implicated in both the initiation and the maintenance of the transformed state (2, 24, 35, 39). Both the large-T antigen and the small-t antigen are expressed in productively infected as well as transformed cells (1–3, 5, 8, 35, 39, 43, 52). Temperature-sensitive *A* mutants map in the early region of the SV40 genome that codes for the large-T antigen (27, 28). The mutant-transformed cells require high concentrations of serum upon their shift to restrictive temperature from permissive temperature (35), indicating that the large-T antigen is continually required for the ability of transformed cells to grow in low concentrations of serum.

A class of SV40 viable early deletion mutants with deletions ranging between 0.54 and 0.59 map units on the SV40 genome produces wild-type large-T antigen and goes through the lytic cycle in permissive cells with the same efficiency as does wild-type SV40 (7, 51, 52). Therefore, the region that maps between 0.54 and 0.59 map units on the SV40 genome is not essential for the replication of SV40 to occur. However, Sleigh et al. (52) have shown that these deletion mutants of SV40 have a reduced ability to transform mouse, rat, and rabbit cells. Martin et al. (36) have shown that the transformation frequency of Chinese hamster lung cells infected during a resting state with viable early deletion mutants of SV40 is much lower than the transformation frequency of the wild-type virus-infected cells. Graessmann et al. (11) have recently shown that the small-t gene function of SV40 is required for the loss of actin cables in rat embryonic fibroblast cells. Thus, the small-t gene function of

SV40 seems to have a role in the establishment of transformation under certain growth conditions.

During productive infection of AGMK cells with SV40, cellular DNA, RNA, and protein syntheses increase 8- to 10-fold (6, 12, 25, 38, 47). We have shown previously that the infection with SV40 of TC7 cells, a subline of AGMK cells, stimulates the appearance of a host cell centriolar antigen which has been localized in the centriolar region of TC7 cells by indirect immunofluorescence microscopy (15). We have also shown that the centriolar antigen consists of two polypeptides of 14,000 and 17,000 daltons (16). The centriolar antigen is stimulated after SV40 infection and is observed before the appearance of T antigen, an early gene product of SV40 (15). By using various temperature-sensitive mutants of the complementation groups A, B, C, BC, and D, we have demonstrated that neither the large-T gene function nor the structural genes of SV40 are required for the stimulation of the centriolar antigen (16). However, by using the early viable deletion mutants of SV40, *dl*2004 and *dl*2006, we have demonstrated that the sequences mapping between 0.54 and 0.59 map units on the SV40 genome are essential for the stimulation of the host antigen (16). Thus, the small-t gene function of SV40 is essential for the stimulation of the centriolar antigen. An antigenically related polypeptide has been identified in the centriolar region of many other eucaryotic cells of different origin (33). A related antigen is also present in the basal body region of differentiated multiciliated cells from many different species and has been localized near the basal body region of ciliated cells from cat trachea by immunoelectron microscopy (33).

We have shown previously that de novo protein synthesis from 0 to 5 h after infection is essential for the stimulation of the centriolar antigen in TC7 cells (16). In the present paper, we show that de novo RNA synthesis is also necessary for the stimulation of the host antigen. We also extend our previous studies with deletion mutants to show that an intact small-t gene function of SV40 is essential for the host cell antigen stimulation. In this paper, we also demonstrate that the nondefective adenovirus-SV40 hybrid viruses, $Ad2^+ND1$, $Ad2^+ND2$, $Ad2^+ND3$, $Ad2^+ND4$ and $Ad2^+ND5$, which lack the wild-type SV40 small-t gene sequence, also stimulate the host cell centriolar antigen to the same extent that wild-type SV40 does. Thus, a gene function similar to the small-t gene function of SV40 is present in adenovirus that can stimulate the centriolar antigen in TC7 cells.

MATERIALS AND METHODS

Cells and viruses. TC7 cells, a subline of AGMK cells, were grown on glass cover slips (22 by 22 mm; Gold Seal, Clay Adams) in petri dishes, using Dulbecco modified Eagle medium supplemented with 0.3 mg of arginine hydrochloride per ml, 1.5 mg of glutamine per ml, 20 μg of histidine hydrochloride per ml, and 5 mg of glucose per ml containing 10% (vol/vol) fetal calf serum. Subconfluent cells were infected with wild-type SV40 (strain 341), deletion mutants of SV40, adenovirus-SV40 hybrid viruses, or adenovirus 2 at a multiplicity of infection of about 5 to 10. The SV40 deletion mutants used in these studies were *dl*2000, *dl*2001, *dl*2003, *dl*2005, and *dl*2007 (52). The adenovirus-SV40 hybrid viruses used were $Ad2^+ND1$, $Ad2^+ND2$, $Ad2^+ND3$, $Ad2^+ND4$, and $Ad2^+ND5$ (30, 31). Infected cells were cultured in a medium containing 3% fetal calf serum. Both infected and uninfected cells were cultured at 37°C in moisturized chambers with a 10% CO_2 level. When actinomycin D was used, cells were infected on cover slips as described above, and at various times after infection, the cover slips were transferred to fresh media plus 3% fetal calf serum containing actinomycin D at 5 μg/ml (Calbiochem). Incubation was continued in the presence of actinomycin D for up to either 8 or 20 h after infection, at which time the cells were fixed as described below.

Fixation, staining, and immunofluorescence analysis of samples. Cover slips were removed from the cultures at various times after infection, rinsed in phosphate-buffered saline (10 mM sodium phosphate [pH 7.2], 0.15 M NaCl), fixed in a 1:1 mixture of acetone and methanol (−20°C) for 5 min, fixed again in acetone and methanol for 5 min, and air dried. These cover slips were stored at −20°C for later use. Uninfected cover slips were processed in a similar manner. The cover slips were overlaid with 40 μl of appropriately diluted antiserum (15) and incubated for 60 min at 37°C. The cover slips were rinsed well in phosphate-buffered saline and stained with 40 μl of fluorescein-conjugated goat anti-rabbit immunoglobulin G (National Cancer Institute) at a 1:25 dilution with phosphate-buffered saline. The cover slips were incubated at 37°C for another 60 min, rinsed several times in phosphate-buffered saline, and mounted on a drop of Gelvatol (Monsanto Co.).

The cells were observed with a Leitz Orthoplan microscope with 40 and 100× objectives and were photographed with a Vario-orthomat camera on Kodak Tri-X film. An Olympus micrometer was used to determine the magnification factor.

RESULTS

Stimulation of host cell centriolar antigen by SV40. We have shown previously (15) that infection of TC7 cells with SV40 results in the stimulation of a host cell antigen at the centriolar region. This antigen may be visualized by indirect immunofluorescence, using preimmune or immune sera. The brightly staining circular structure in the fluorescence micrograph (Fig. 1d) corresponds to the dense structure seen near the nucleus in the phase micrograph (Fig. 1c). Uninfected cells also exhibited positive staining in this region (Fig. 1a), though at a much lower frequency, indicating that SV40 was functioning to stimulate a natural host antigen. In a randomly growing population of uninfected cells, the

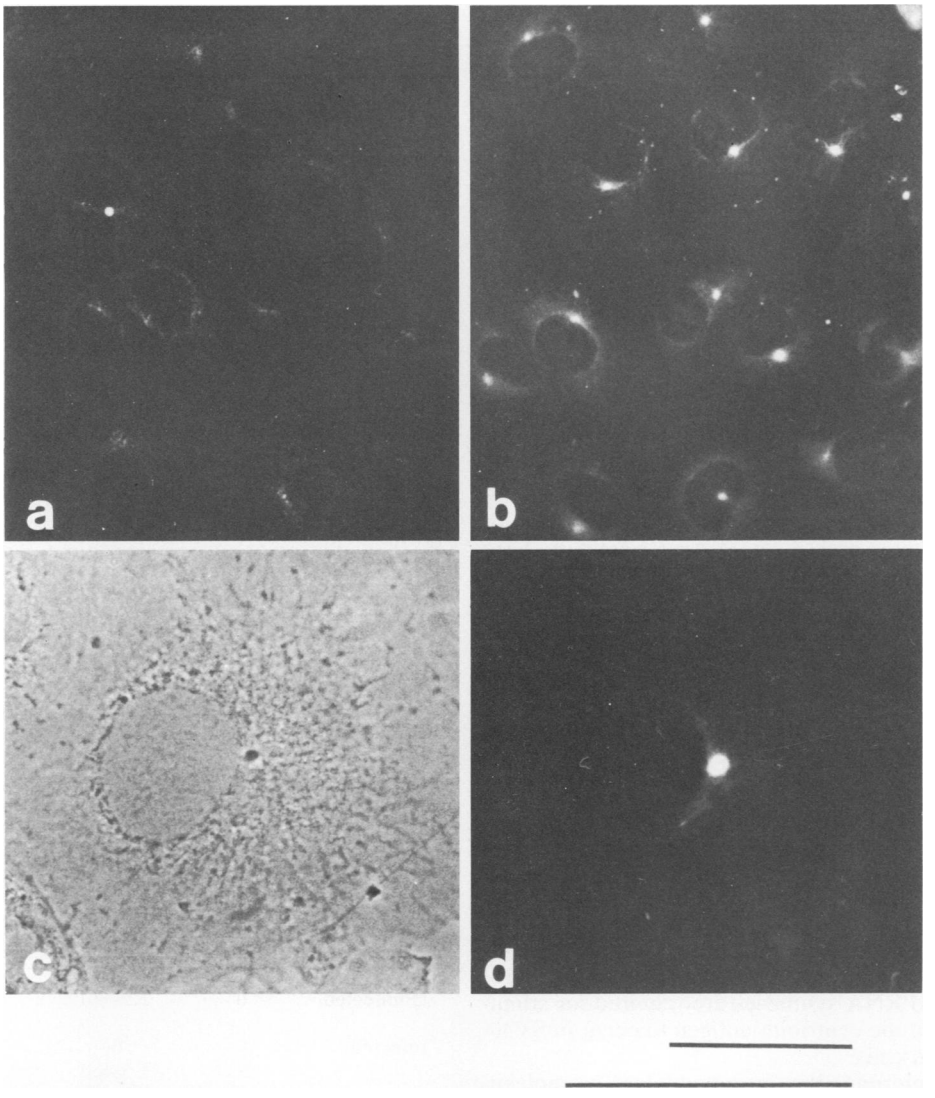

FIG. 1. Fluorescence micrographs and phase micrograph of stained cells. Shown are fluorescence micrographs of uninfected (a) and infected (b) cells and phase-contrast (c) and fluorescence (d) micrographs of an infected cell. Upper bar = 60 μm (a and b); lower bar = 50 μm (c and d). Cells were cultured, fixed, and stained as described in the text. Infected cells were fixed at 20 h after infection with SV40. Note that only one of 15 uninfected cells shows staining in the centriolar region (a), whereas all infected cells show the staining (b). The bright staining seen in the fluorescence micrograph (d) corresponds to the dense structure seen in the phase-contrast micrograph (c) of the same cell.

incidence of positive centriolar antigen staining is on the order of 5 to 20%. Upon infection with SV40, the frequency of cells expressing positive staining for the antigen rises to approximately 80% at 24 h after infection (Fig. 1b). In general, maximum positive staining is observed at 20 h after infection. The amount of stimulation that was seen appeared to be affected by the input virus multiplicity of infection. When cells were infected at a lower multiplicity of infection (0.2 PFU per cell), the frequency of positive centriolar antigen staining observed at 20 h after infection was about 30%. When the multiplicity of infection was increased 10-fold, there was an obvious 2-fold increase in the centriolar antigen staining, though the frequency of positive centriolar staining did not increase linearly with increasing virus multiplicity of infection. It is possible that there is another cellular factor(s) that is responsible for the stimulation of centriolar antigen staining in TC7 cells. We have also shown that stimulation occurs at wild-type levels for temperature-sensitive mutants of the SV40 A, B, C, BC, and D complementation

groups (16). Therefore, SV40-specific stimulation of the host centriolar antigen occurred independently of the large-T or structural genes of SV40.

De novo protein and RNA syntheses for centriolar antigen stimulation. The increased frequency of centriolar staining seen upon infection with SV40 could be due to de novo synthesis of the antigen or to a reorganization of preexisting antigen molecules. If the increase occurs via a reorganization event, it should not be affected by the addition of a protein or RNA synthesis inhibitor. However, if de novo protein or RNA synthesis is essential for stimulation, the addition of such inhibitors should prevent the induction event.

Cells were infected on cover slips as described above, and at various times after infection they were transferred to medium containing either the RNA synthesis inhibitor actinomycin D or the protein synthesis inhibitor puromycin. After continued incubation in the presence of the inhibitor, cells were fixed and scored for the presence of the centriolar antigen. Uninfected cells show a frequency of positive centriolar antigen staining of about 8% (Table 1). When infected with wild-type SV40 in the absence of actinomycin D, this frequency rose to between 29 and 47%. Variation in the degree of antigen stimulation seemed to be a result of variations in the virus titer. When cells were infected and transferred at 1.5 h after infection to medium containing actinomycin D, uninfected levels of the antigen persisted for as long as 20 h after infection (Table 1). We have previously reported similar results, using puromycin as an inhibitor of protein synthesis (16). Therefore, both protein and RNA syntheses are required for stimulation of the centriolar antigen to occur in SV40-infected cells.

To determine the time at which macromolecular synthesis was essential for stimulation of the host cell antigen, actinomycin D was added to infected cells at various times after infection. The frequency of centriolar staining was then assayed at either 8 or 20 h after infection. When actinomycin D was added to infected cells at times up to and including 4.5 h after infection, no stimulation of the antigen by SV40 was seen when it was assayed at either 8 or 20 h after infection (Table 1). The addition of actinomycin D at times after 4.5 h after infection did not inhibit the stimulation of the centriolar antigen by SV40. Infected cells which received actinomycin D at these later times showed an increase in the frequency of positive centriolar staining which was similar to, although slightly lower than, the frequency of staining seen in infected cells in the absence of the inhibitor. This increase suggested that the infected cells were responding to an SV40-specific signal within the period between infection and the addition of inhibitor. The slightly lower antigen levels seen when the inhibitor was added at times after 5 h of infection suggested that some additional stimulation of the antigen would normally be occurring at these later times. We have previously demonstrated a similar effect when infected cells are exposed to the protein synthesis inhibitor puromycin (16).

These results indicate an absolute requirement for RNA synthesis for SV40-specific stimulation of the centriolar antigen to occur. Therefore, stimulation of the antigen was not the result of a simple reorganization event. The time at which synthesis was required was specific, and it occurred primarily within the first 4.5 to 5 h after infection. Inhibition of either protein or RNA synthesis at later times had no effect on the antigen stimulation. We have shown previously (16) that addition of puromycin at times up to 9 h after infection inhibits the synthesis of the viral large-T antigen. Therefore, SV40-specific stimulation of the centriolar antigen appears to precede the expression of large-T antigen, the earliest known viral gene product.

Small-t gene function of SV40 and stimulation

TABLE 1. Frequency of positive centriolar antigen staining in SV40-infected cells treated with actinomycin D[a]

Expt	Total h of infection	Time in actinomycin D (h postinfection)	Positive centriolar staining (%)
A			
Uninfected	0	1.5–8.0	7
Infected	8	0	29
	8	1.5–8.0	7
	8	3.0–8.0	7
	8	4.5–8.0	6
	8	6.0–8.0	13
	8	7.5–8.0	33
B			
Uninfected	0	1.5–20.0	8
Infected	20	0	29
	20	1.5–20.0	8
	20	3.0–20.0	7
	20	4.5–20.0	6
	20	6.0–20.0	20
	20	7.5–20.0	21

[a] Cells were cultured and infected with wild-type SV40 as described in the text. At the indicated times, actinomycin D was added, and incubation continued until 8 (experiment A) or 20 (experiment B) h after infection, at which time cells were fixed and stained as described in the text.

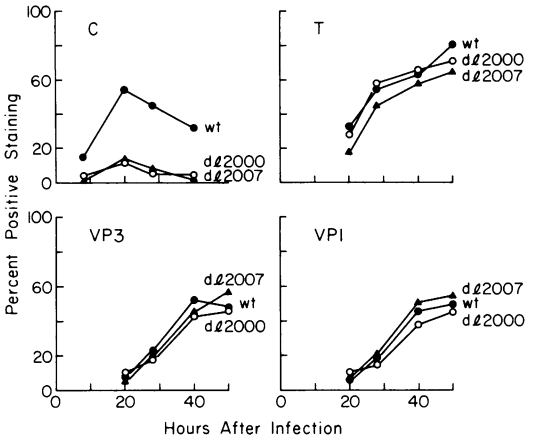

FIG. 2. Expression of various antigens in wild-type (wt)- and deletion mutant (*dl*2000 and *dl*2007)-infected TC7 cells. TC7 cells were infected with either wild-type SV40 or early viable deletion mutants, fixed at various times after infection, reacted with anti-T, anti-VP3, or anti-VP1 serum, and examined for the expression of each specific antigen. (C and VP3) Results from cells reacted with anti-VP3 serum in which distinct staining in the centriolar region (Fig. 1) and the nuclear region (14), respectively, was observed; (T) T-antigen-positive nuclear staining; (VP1) positive cytoplasmic or nuclear staining or both after reaction with appropriate serum (14). In each case, about 300 cells were examined.

of centriolar antigen. We have shown previously that the small-t gene region of wild-type SV40 is essential for the centriolar antigen stimulation (16). Earlier in our studies, we used the early viable deletion mutants of SV40, *dl*2004 and *dl*2006, with deletions mapping between 0.54 and 0.59 map units on the SV40 genome. Both of these mutants fail to stimulate centriolar antigen to the levels of wild-type-infected cells. In the present paper, we extend our previous studies to various other deletion mutants of the same class which have a deletion in the small-t coding region. During lytic infection, these mutants are known to synthesize an intact large-T antigen at wild-type levels (20, 52).

We have examined the stimulation of centriolar antigen and the production of large-T antigen and viral polypeptides VP1 and VP3 by the deletion mutants *dl*2000 and *dl*2007 and by wild-type SV40. The levels of T antigen, VP1, and VP3 in the deletion mutant-infected cells were comparable to those of wild-type-infected cells, as expected (Fig. 2). However, the deletion mutants failed to stimulate the centriolar antigen above the level of uninfected cells. Early viable deletion mutants with deletions mapping between 0.54 and 0.59 map units on the SV40 genome either produce an altered small-t protein or fail to produce small-t protein (20, 52). Table 2 summarizes the results obtained with various deletion mutant-infected TC7 cells. In cells infected with deletion mutants, the incidence of staining in the centriolar region remained at the same level as that of uninfected TC7 cells throughout the course of infection. However, the stimulation of the centriolar antigen in wild-type-infected cells increases up to 20 h after infection, after which time it declines as infection progresses (15). Thus, the small-t mutants *dl*2000, *dl*2001, *dl*2003, *dl*2004, *dl*2005, *dl*2006, and *dl*2007 are defective in the stimulation of the host centriolar antigen. This indicates that an intact, functional small-t antigen is required for the stimulation of centriolar antigen in TC7 cells.

Centriolar antigen stimulated in cells infected with nondefective adenovirus-SV40 hybrid viruses. To see whether the failure of stimulation of the centriolar antigen by viable early deletion mutants could be overcome by specific gene functions of other viruses, we used adenovirus-SV40 hybrid viruses to infect TC7 cells. These nondefective adenovirus-SV40 hybrid viruses,

TABLE 2. Frequency of centriolar antigen staining in cells infected with SV40 early viable deletion mutants, adenovirus-SV40 hybrid viruses, or adenovirus 2[a]

Cells infected with:	% of cells showing positive centriolar antigen staining at various times after infection				
	4 h	8 h	12 h	24 h	36 h
Uninfected[b]					
Wild-type 341	16	19	68	63	46
*dl*2000	4	4	15	1	2
*dl*2001	12	13	19	18	
*dl*2003	13	11	19	19	
*dl*2005	13	9	16	15	
*dl*2007	14	1	11	12	2
Ad2$^+$ND1	48	62	55	47	
Ad2$^+$ND2	41	38	45	50	38
Ad2$^+$ND3	30	56	58	35	
Ad2$^+$ND4	35	51	62	54	
Ad2$^+$ND5	43	51	48	75	47
Adenovirus 2	31	16	15		

[a] Cells were cultured, infected, fixed at different times after infection, and stained as described in the text. The deletion mutants used were *dl*2000, *dl*2001, *dl*2003, *dl*2005, and *dl*2007. The adenovirus-SV40 hybrid viruses used were Ad2$^+$ND1, Ad2$^+$ND2, Ad2$^+$ND3, Ad2$^+$ND4, and Ad2$^+$ND5. The frequency of staining in the centriolar region was obtained by examination of about 300 cells for each data point.

[b] The number for uninfected cells, 10 to 16%, represents the frequency of centriolar antigen staining observed in uninfected cells in several different experiments.

$Ad2^+ND1$, $Ad2^+ND2$, $Ad2^+ND3$, $Ad2^+ND4$, and $Ad2^+ND5$, have a deletion in the adenovirus 2 genome in a region that is not essential for adenovirus growth in human or monkey cells (30, 31). They also have an insertion of a portion of SV40 DNA which can provide a helper function for the growth of these viruses in monkey cells (30, 31). The inserted SV40 sequences are of various lengths, and they all begin at 0.11 map units on the SV40 genome (17, 29). Similarly, the deleted adenovirus sequences are of different lengths, but they all end at a common point (85.5 map units) in the adenovirus genome. Of the nondefective hybrid viruses described above, only $Ad2^+ND4$ contains part of the coding sequences for SV40 small-t antigen mapping between 0.11 and 0.59 map units on the SV40 genome. If a particular gene function of adenovirus does not complement the small-t gene function of SV40, then the stimulation of centriolar antigen should not be observed in TC7 cells infected with adenovirus-SV40 hybrid viruses. On the other hand, if the lack of the small-t gene function of SV40 is complemented by some adenovirus gene(s), the centriolar antigen should be stimulated to the level of wild-type-infected cells.

All of the hybrid viruses described above stimulated the centriolar antigen to the same levels as did wild-type SV40 (Table 2). The percentage of positive staining increased up to about 24 h after infection in both wild-type- and adenovirus-SV40 hybrid virus-infected cells. The frequency of staining decreased after 24 h as infection progressed. Therefore, adenovirus possesses a gene function that can substitute for the SV40 small-t gene function in the stimulation of the centriolar antigen in TC7 cells.

TC7 cells are semipermissive for human adenoviruses. Infection of TC7 cells with adenovirus also resulted in the stimulation of centriolar antigen (Table 2). The duration of the stimulation observed in adenovirus-infected TC7 cells was different from that seen in SV40-infected cells. The percentage of positive staining seen in the centriolar region increased up to about 4 to 5 h after infection and decreased thereafter (Table 2). Similar results were obtained in two other experiments. The highest frequency of the stimulation observed in adenovirus-infected TC7 cells was at about 4 h of infection, whereas in cells infected with wild-type SV40 or adenovirus-SV40 hybrid viruses, the highest frequency was observed at about 20 h of infection. Although there may be another factor that influences semipermissive host-virus interaction, either cellular or viral, the observation that adenovirus stimulates centriolar antigen in TC7 cells agrees with the result described above and indicates that adenovirus has a gene function that can stimulate the centriolar antigen in TC7 cells.

DISCUSSION

The infection of TC7 cells by SV40 stimulates certain host cell antigens which are localized in the centriolar region (16). De novo syntheses of both protein (16) and RNA, which occur within the first 5 h after infection, are essential for the stimulation of this antigen by SV40. Viable early deletion mutants that map between 0.54 and 0.59 map units on the SV40 genome have been shown to have reduced transforming ability (7, 51, 52). Although these viable early deletion mutants, with the exception of *dl*2003, are tumorigenic in vivo, they have a longer latency period and a lower tumor incidence compared with the wild-type virus (32, 54). These mutants do not stimulate the centriolar antigen above the level of uninfected cells, indicating that an intact small-t gene function is essential for the stimulation of the centriolar antigen in SV40-infected TC7 cells.

The small-t gene function of SV40 is known to have a role in the transformation of cells. The transformed state of a cell is related to an alteration of the cytoskeletal structure of the cell which involves the disappearance of organized cytoplasmic actin cables (41). When 3T3 cells are transformed by SV40, a loss of actin-containing sheaths is seen (41). Host range transformation mutants that map in the early region of polyoma virus and are defective in transformation have been described (13). These host range transformation mutants of polyoma virus do not alter the cellular morphology and actin cables after infection of rat fibroblasts, whereas wild-type polyoma does (50). When rat embryonic fibroblast cells were microinjected with SV40 DNA fragment that contained the entire coding region of small-t antigen, the cells exhibited a loss of actin cables (11). However, microinjection of cells with SV40 DNA lacking the region between 0.54 and 0.59 map units did not result in the loss of actin cables. Cell morphology and cell movement depend upon the organization of cytoskeletal components. Cytoplasmic microtubules, microfilaments, and intermediate filaments are the major components of cytoskeleton. Thus, cell morphology changes when the cytoskeleton of a cell is disrupted. It is of interest that the small-t gene function of SV40 which is required for the loss of actin cables and is known to be involved in the transformation of cells is also essential for the stimulation of centriolar antigen in TC7 cells. Perhaps the small-t gene function is required to alter the cell growth property in infected cells. The inability of viable early deletion mutants to stimulate the host centriolar antigen is a result of failure to

alter the growth property of the cell due to the lack of an intact small-t gene function. No stable cytoplasmic 19S small-t mRNA is made by the early viable deletion mutants of SV40 that lack the proximal splice junction (20). Shortened small-t mRNAs are made by the SV40 deletion mutants that do not involve splice junction, suggesting that the primary sequence of the splice junction and the secondary or tertiary structure or both of RNA regulates the location and frequency of the splicing event (20). Our studies suggest that in cells where shortened small-t polypeptides are made either in the normal reading frame or by frame shift reading, the shortened or altered small-t sequences are not sufficient for the induction of the centriolar antigen.

The centriolar antigen is also stimulated in TC7 cells infected with nondefective adenovirus-SV40 hybrid viruses. Adenovirus can be grown in monkey cells by coinfection with SV40 or by insertion of a portion of SV40 genome into the adenoviral genome (44). Several nondefective hybrid viruses derived from adenovirus 2 stock contain a part of SV40 genome inserted into the adenovirus genome (30, 31). Among the nondefective adenovirus-SV40 hybrid viruses, $Ad2^+ND1$, $Ad2^+ND2$, $Ad2^+ND3$, $Ad2^+ND4$, and $Ad2^+ND5$, only $Ad2^+ND4$ contains a part of the coding sequences for the small-t protein of SV40. This sequence, mapping between 0.11 and 0.59 map units of SV40, is not sufficient to code for an intact SV40 small-t protein in adenovirus-SV40 hybrid viruses. All of the adenovirus-SV40 hybrid viruses described above stimulate the host cell antigen in the centriolar region to the same extent as does wild-type SV40. The centriolar antigen can also be stimulated in TC7 cells after their infection with adenovirus 2. Thus, the adenovirus genome seems to have a similar function in the centriolar antigen stimulation in TC7 cells. The precise sequence of the adenovirus genome that complements the small-t gene of SV40 for the stimulation of centriolar antigen in TC7 cells has not yet been determined. It should be possible to localize this region precisely with the use of various adenovirus mutants. However, it is evident from the above studies, in which the adenovirus-SV40 hybrid viruses were used for the stimulation of centriolar antigen, that the sequences that map between 78.7 and 85.5 map units on the adenovirus genome, including early region 3, are not involved in complementing the small-t gene function of SV40 for the centriolar antigen stimulation. When semipermissive AGMK cells are infected with adenovirus, T antigens are expressed and viral DNA replication takes place; however, the adenovirus late proteins are not expressed (9, 26, 37). The adenovirus T antigens are coded for by the left-hand 14% of the adenovirus genome. Thus, the adenovirus gene function which is responsible for the stimulation of centriolar antigen in TC7 cells and can complement the lack of small-t gene function of SV40 for the stimulation is likely to be one of the genes that codes for the adenovirus T antigens.

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