Mutational Analysis of Simian Virus 40 T Antigen: Isolation and Characterization of Mutants with Deletions in the T-Antigen Gene

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A series of mutants of simian virus 40 has been constructed with deletions in the coding sequence for large T antigen. Nucleotide sequence analysis indicates that 4 mutants have in-phase and 11 have out-of-phase deletions. Mutant DNAs were assayed for the following activities: the ability to form plaques, the ability to produce T antigen as scored by indirect immunofluorescence, viral DNA replication, and morphological transformation of rat cells. Two viable mutants were found, and these had deletions confined to the carboxyl terminus of T antigen. Only those mutants coding for polypeptides greater than 40% of the length of wild-type T antigen produced detectable nuclear fluorescence. The two viable mutants with deletions in the carboxyl terminus of the protein retained the ability both to replicate their DNA, although at a reduced level, and to transform nonpermissive cells. Mutants with sequence changes that result in the loss of more than 117 amino acids from the carboxyl terminus were not viable and were also defective in the DNA replication and transformation functions of T antigen, although several produced detectable nuclear fluorescence. These functions were also sensitive to the removal of amino acids near the amino terminus and in the middle of the protein.

The major protein expressed early in simian virus 40 (SV40) infection of either permissive monkey cells or nonpermissive rodent cells is the large T antigen. T antigen is a multifunctional protein of approximately 82,000 daltons for which a number of biological and biochemical activities have been described. This protein is required for the initiation of viral DNA replication (79), stimulation of cellular DNA synthesis (7, 18, 31, 32, 68, 84), increase in host transcriptional activity in SV40-infected cells (45, 52, 57, 66), and SV40 human adenovirus helping function (16, 35, 59). In addition, T antigen negatively regulates early region transcription (1, 34, 62, 82) and may positively regulate the late region gene expression (1, 11, 34, 54, 65). The initiation, as well as the maintenance, of the transformation phenotype of SV40-transformed cells requires the continuous expression of T antigen (2, 5, 36, 37, 43, 53, 77, 80), and the specific transplantation antigen present in such cells appears to be related to T antigen (21, 88).

Several biochemical activities of T antigen have been described. Purified T antigen binds specifically to a region of the viral DNA which includes the origin of DNA replication (47, 48,

61, 69, 83) and transcriptional start sites (19, 20, 27, 28, 60). Genetic and biochemical evidence indicates that the DNA binding activity of T antigen is related to viral DNA replication (12, 51, 72, 74) and regulation of early region transcription (13, 29, 51, 64). Other biochemical activities so far ascribed to T antigen are an ATPase activity (85) and the ability to complex with a 53,000- to 55,000-dalton host protein (40, 41). As yet no correlation has been made between the ATPase and host protein binding activities and any biological property of T antigen.

Several possibilities exist as the basis for the multiple activities of T antigen. T antigen may have a single activity or target in the infected cell which may trigger a series of events resulting in the observed multiple biological activities (89). Another possibility is that multiple functional forms of T antigen exist in the cell, each differing by a single or series of post-translational modifications which alter the activity of the peptide. In fact, T antigen is known to be *N*-acetylated at the amino terminus (49), phosphorylated (81), and ADP ribosylated (23); the biological significance of these modifications is not yet known. Finally, T antigen may be organized as a series

of domains with different regions of the polypeptide encoding different biochemical activities. Indications that T antigen consists of independent functional domains stem from several sources. The human adenovirus helping function of T antigen has been shown to reside in the carboxyl-terminal 70 amino acid residues which are necessary and sufficient for this activity (10, 14), and microinjection of restriction enzyme-generated DNA fragments has suggested that the amino-terminal three quarters of the protein is sufficient for the stimulation of cellular DNA synthesis (18, 50).

Our approach to dissecting the various activities of T antigen has been to construct by *in vitro* mutagenesis mutants which affect the structure of T antigen and to test these mutants for each of the biological or biochemical activities of T antigen. By correlating the activity of each mutant with the structure of the protein it produces, we hope to determine whether T antigen has distinct functional domains. In this paper we report the structure of mutant genomes with deletions in the T-antigen gene. These mutants are of two types: those with in-phase deletions that result in the loss of specific segments of T antigen and those with out-of-phase deletions that generate an overlapping set of T-antigen polypeptides extending from the amino terminus of the protein. Three of the biological activities associated with T antigen have been studied in cells infected with mutant DNA: plaque formation on lawns of permissive cells in the presence or absence of a helper virus, replication of viral DNA, and morphological transformation of non-permissive cells. In the accompanying paper (75) the mutants are examined for their ability to stimulate cellular DNA synthesis and activate silent rRNA genes.

MATERIALS AND METHODS

Cells and viruses. The small-plaque strain of SV40 was propagated in BSC40 cells, and viral DNA was prepared as described earlier (3, 4). The viability of mutants was determined by plaque formation on BSC40 cell monolayers.

Construction of deletion mutants. The method of construction of SV40 deletion mutants has been described previously (55, 56). Initially, mutants with deletions in the early gene segment were constructed in SV40 DNA and propagated in monkey cells by using a late deletion mutant, *dl1007*, as a helper (56). These mutant DNAs have since been molecularly cloned in pBR322, via their *Bam*HI sites, and this cloned DNA has been used in all of the experiments described in this report. The remaining deletion mutants were constructed in the pBR322/SV40 recombinant plasmid pSVB3 and cloned and propagated in *Escherichia coli* (55).

Preparation of plasmid DNA. Recombinant plasmid DNAs were propagated in either HB101 or MM294 cells. After overnight amplification in the presence of

150 to 170 μ g of chloramphenicol per ml (8), plasmid DNA was extracted using a cleared-lysate technique (33; K. W. C. Peden, manuscript in preparation) and purified by twice banding in ethidium bromide-CsCl gradients. The DNA was dialyzed against 10 mM Tris-hydrochloride-1 mM EDTA, pH 8.0.

DNA sequencing. DNA sequencing of mutational sites was carried out by the method of Maxam and Gilbert (44), using the thin gels of Sanger and Coulson (67). Appropriate restriction fragments were labeled at the 5' ends with 32 P by the polynucleotide kinase reaction or at the 3' ends by filling in 5' overhangs by incubating with an α - 32 P-deoxynucleoside triphosphate and DNA polymerase I from *Micrococcus luteus*.

Complementation tests. Complementation tests were carried out by coinfecting BSC40 cell monolayers with mixtures of 1.5 ng of excised and ligated mutant DNA and 50 ng of either *tsA28* DNA or *dl1007* DNA per 5-cm dish by the DEAE-dextran method (46). Plaques were counted after 12 days at 40°C.

Indirect immunofluorescence in monkey cells. BSC40 cells in chamber slides were infected with 100 ng of plasmid DNA by the DEAE-dextran method (46). After 24 h the cells were fixed in cold acetone and stained with hamster SV40 anti-T serum (58).

DNA replication in monkey cells. DNA replication of the mutants was measured in BSC40 cells as described by Peden et al. (55). Mutant DNAs were first excised from the recombinant plasmids with *Bam*HI, and the viral DNA was recircularized by ligation with T4 DNA ligase at DNA concentrations of between 1 and 2 μ g/ml. This DNA was used to infect subconfluent BSC40 monolayer cultures in 50-mm petri dishes by the DEAE-dextran method (46). Mutant DNA (25 ng of the original plasmid DNA per dish) alone or mutant DNA (25 ng) plus complementing late deletion *dl1007* helper DNA (25 to 100 ng) was used. At various times after infection, the cells were washed once with phosphate-buffered saline containing 0.5 mM EDTA and lysed by the addition of 0.5 ml of Hirt (30) lysing solution (0.6% sodium dodecyl sulfate, 10 mM EDTA, pH 7.5). The lysate was transferred to 1.5-ml polypropylene Eppendorf microfuge tubes containing 150 μ l of 5 M NaCl. The tubes were inverted several times and placed at 4°C overnight or longer. Centrifugation for 10 min in an Eppendorf microcentrifuge (type 5412) was sufficient to pellet the precipitates. The supernatants were removed, made 0.5% in sodium dodecyl sulfate and 10 μ g/ml in proteinase K, and digested for at least 4 h at 37°C. The DNA was purified further by phenol extraction, phenol-CHCl₃-isoamyl alcohol (25:24:1) extraction, and CHCl₃-isoamyl alcohol (24:1) extraction and concentrated by isopropanol precipitation and centrifugation. After washing the precipitates with 95% ethanol and drying them under vacuum, the DNA was dissolved in 100 μ l of 0.1 mM EDTA, pH 7.5. Five microliters of each sample was digested with *Dpn*I (to digest the input DNA) and *Bcl*I (to linearize the replicated DNA), fractionated on 1% agarose gels, and transferred to nitrocellulose filters by the Southern (76) method. After transfer, the filters were rinsed in 2 $\times$ SSC (1 $\times$ SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate) and dried at room temperature. Hybridization with SV40 [32 P]DNA (labeled *in vitro* by nick translation [42, 63]) and washing of filters will be described elsewhere (K. W. C. Peden, manuscript

in preparation). Autoradiographic exposures were with Kodak XS or Cronex 6-plus film without intensification.

Transformation of rat embryo fibroblasts. Rat embryo fibroblasts (REF 52), kindly supplied by W. Topp, Cold Spring Harbor Laboratory, were seeded into 5-cm dishes at approximately $5 \cdot 10^4$ cells per dish the day before infection. The calcium phosphate procedure of Graham and van der Eb (24), modified to include a glycerol shock (17), was used for transfection of the mutant DNAs. In a typical experiment, four dishes were transfected with 1 to 2 μ g of mutant DNA per dish as a recombinant plasmid, and the following day one dish from each infection was trypsinized and replated into three or four 5-cm dishes. Dishes were fed every 3 to 4 days with minimal essential medium plus 10% fetal bovine serum, and foci were scored at 2 and 3 weeks after infection.

RESULTS

Construction and isolation of mutants. Mutants of SV40 with deletions in the T-antigen gene were constructed and isolated as described earlier (55, 56). Since we wanted a collection of mutants with deletions throughout the T-antigen gene, some of which would be in phase and others out of phase, we used random cleavage of SV40 DNA or a pBR322/SV40 recombinant molecule as the starting material for the mutagenesis procedure. In brief, form I SV40 or plasmid pSVB3 DNA was nicked once with DNase I in the presence of ethidium bromide (25), the nick was converted to a small gap with DNA polymerase I of *M. luteus* (71), and the gapped

molecules were converted to linear molecules with S1 nuclease. These randomly linearized molecules were then ligated to circles. When SV40 DNA was the starting material, the ligated DNA was used to infect BSC40 cells together with DNA of the late deletion mutant *dl1007* (38), and complementation plaques were analyzed (4, 73). When the recombinant plasmid pSVB3 was the starting material, the ligated DNA was used to transform *E. coli*, and individual colonies were analyzed. In each case mutant DNAs were identified by alterations in their restriction enzyme digestion patterns. Finally, all mutants of interest that were isolated originally in BSC40 cells were cloned in *E. coli* as pBR322 recombinants.

Figure 1 shows the distribution of early region mutants isolated by the above procedures. In addition, five mutants were found to have deletions in the late genes and were not studied further. Fifteen mutants have deletions in the T-antigen gene, of which three are in regions common to small- and large-T-antigen coding sequences. The two remaining mutants recorded in Fig. 1 (*dl1134* and *dl1141*) have deletions of noncoding regions of the genome adjacent to the T-antigen gene.

DNA sequence analysis of mutant DNAs. Most of the T-antigen mutants shown in Fig. 1, as well as *dl1134* and *dl1141*, were selected for sequence analysis by the Maxam-Gilbert method, generally by 3' labeling of a restriction site near one end of the deletion. The results are summarized in

FIG. 1. Map positions of deletions in SV40 T-antigen mutants. The extent and location of each deletion, indicated by the horizontal lines, were determined by nucleotide sequencing. Deletion mutant numbers are given on the vertical axis, and map coordinates are given on the horizontal axis. The sequences coding for the large and small tumor antigens are indicated. Arrowheads indicate the presence of inserted nucleotides.

FIG. 2. Structure of mutant genomes with their predicted amino acid sequences. The nucleotide sequence for each mutant is presented. In each case the number of nucleotides deleted is given within parentheses in base pairs (bp), with the numbers of the last nucleotide before the deletion and the first nucleotide after the deletion indicated. The numbering system is that given by Tooze (87). Underlined nucleotides represent the insertion of additional sequences. Underlined amino acids represent missense amino acids resulting from frame shifts.

Table 1 and in Fig. 2 together with the predicted changes in amino acid sequences of T antigen. Although all of the mutants isolated in bacteria had simple deletions of sizes ranging from 30 to greater than 800 base pairs, some of those isolated in monkey cells with a helper were found to have insertions or duplications or both at or near the deletion junctions (*dl1046*, *dl1061*, and *dl1066*). Of the 17 mutants studied, 4 were found to have in-phase deletions in the T-antigen gene (*dl1135*, *dl1047*, *dl1151*, and *dl1140*), and 11 had out-of-phase deletions (*dl1136*, *dl1042*, *dl1137*,

dl1138, *dl1046*, *dl1001*, *dl1055*, *dl1139*, *dl1058*, *dl1061*, and *dl1066*). It should be noted that *dl1136* is missing both T-antigen splice junctions. Of the two mutants with deletions outside the T-antigen coding region, the deletion in *dl1134* removes part of the regulatory sequences preceding the T-antigen gene, whereas that in *dl1141* is between the coding sequences for T antigen and VP1.

Infectivity, complementation, and T-antigen detection. To determine the viability of mutants and whether defects were limited to the T-

FIG. 2. Continued.

antigen gene, each mutant DNA was assayed for plaque-forming ability in BSC40 cells with or without coinfection with a known T-antigen mutant (*tsA28*) or with a VP1, 2, 3 deletion mutant (*dl1007*). At the same time, mutant-infected BSC40 cells were tested for the presence of T-antigen-related polypeptides by indirect immunofluorescence. To carry out these experiments, viral DNA was excised from the plasmid by digestion with *Bam*HI, diluted, and cyclized by treatment with T4 DNA ligase. (Although infectivity tests can be done with linear viral DNA, which is about 1/10 as active as cyclized DNA,

complementation tests were not satisfactory with linear DNA, owing to a variable background of recombination between coinfecting DNAs.) Complementation was considered positive if the number of plaques per nanogram of DNA was at least 1/10 that seen after coinfection by *tsA28* and *dl1007* DNAs, and T-antigen fluorescence was scored as positive if at least 0.1% of cells infected showed fluorescence.

The results of these experiments are summarized in Table 2. The various mutants whose deletions map in the T-antigen gene fall into three groups. The first group is T⁺ (T-antigen

TABLE 1. Map positions and sizes of deletions in SV40 mutants

Deletion no. ^a	Deletion limit ^b	Coordinates	Size of deletions (base pairs) ^c
1135	5114-5082	0.636-0.629	33
1047	4007-3570	0.424-0.341	438
1151	3798-3472	0.385-0.322	327
1140	2792-2763	0.192-0.187	30
1136	5067-4262	0.614-0.461	806
1042	5046-4980	0.623-0.610	67
1137	4453-4423	0.509-0.504	31
1138	4339-4311	0.488-0.482	29
1046	4146-3867	0.451-0.398	280 (25)
1001	4001-3476	0.423-0.322	526
1055	3620-3566	0.351-0.340	55
1139	3557-3526	0.339-0.332	32
1058	3290-3148	0.288-0.260	143
1061	3048-2907	0.231-0.215	142 (14)
1066	2809-2730	0.196-0.181	80 (3)
1134	13-314	0.720-0.662	302
1141	2648-2681	0.171-0.165	34

^a Deletion mutants are grouped according to whether they are in phase or out of phase; the first four are in phase, and the remainder are out of phase.

^b Numbers refer to the nucleotides deleted.

^c Number within parentheses indicates the size of the insertion in base pairs.

nuclear immunofluorescence) and forms plaques without a complementing mutant. Two mutants fall into this class (*dl1066* and *dl1140*), both of which have deletions of codons at the carboxyl-terminal region of the T-antigen gene. Several mutants of this type have been described previously (6, 10, 70). The second group of mutants is T⁺, is nonviable, and complements *dl1007* but not *tsA28*. This class includes *dl1135*, *dl1047*, *dl1001*, *dl1151*, *dl1055*, *dl1058*, and *dl1061*, all of which retain more than about 40% of the functional codons for large T antigen. Presumably, the fluorescence is due to antigenic determinants present on shortened or truncated T antigens (Fig. 1); in some cases this has been demonstrated directly by immunoprecipitation (56). It should be noted that all mutants in this class produce nuclear immunofluorescence, indicating that the shortened T antigens can still reach the nucleus. Another mutant expected to fall into this class by the location of its deletion is *dl1139*. This mutant is T⁺ and nonviable, but is not complemented by either *tsA28* or *dl1007*. By in vitro recombination with wild-type DNA (J. M. Pipas, unpublished data), *dl1139* has been shown to have a second mutation which presumably accounts for its failure to complement *dl1007*. A third group of T-antigen mutants (*dl1042*, *dl1136*, *dl1137*, and *dl1138*) was scored as T⁻, is nonviable, and complements *dl1007* but not *tsA28*. Mutant *dl1136* has a deletion that

removes all splice signals for T-antigen mRNA, and the others have out-of-phase deletions near the amino terminus of the gene. Presumably, the truncated polypeptides produced by these mutants are too short or too unstable to be detected by immunofluorescence. It should be noted, however, that an occasional cell infected with *dl1138* DNA showed positive nuclear fluorescence.

Of the mutants that map in noncoding regions adjacent to the gene for T antigen, one (*dl1134*) does not make detectable T antigen, is nonviable, and does not complement *tsA28* or *dl1007*. These defects are consistent with the loss of both early promoter function and the replication origin. The other mutant (*dl1141*) is T⁺, nonviable, and not complemented by *tsA28* or *dl1007*. Since the mutation deletes a site which is the presumed polyadenylic acid addition signal for late mRNA (15), we assume that it is defective in the formation of late mRNA and therefore does not complement *dl1007*. Because of the unexpected result in the complementation test with *tsA28*, *dl1141* was also tested for its ability to complement each of four early deletion mutants (*dl1135*, *dl1136*, *dl1046*, and *dl1047*) at 37°C. *dl1141* failed to complement any of the mutants; however, after prolonged incubation of cell monolayers, a large number of small plaques were consistently observed. The basis of this unusual property of *dl1141* is not understood.

TABLE 2. Infectivity and T-antigen fluorescence of SV40 deletion mutants

Mutant no.	Infectivity (PFU/ng)			% Nuclei fluorescent
	Mutant alone	+ <i>dl1007</i>	+ <i>tsA28</i>	
<i>tsA28</i>	0	96		ND ^a
1007	0		96	2.4
1135	0	88	2	0.6
1047	0	44	0	0.5
1151	0	63	0	0.3
1140	146	162	104	2.0
1136	0	69	2	<0.1
1042 ^b	3	96	7	ND
1137	0	61	1	<0.1
1138	0	38	2	<0.1
1046	0	60	0	<0.1
1001 ^c	3	24	1	0.4
1055	0	27	0	1.6
1139	0	3	1	1.1
1058	0	56	0	1.3
1061	0	82	3	0.8
1066	108	181	117	2.6
1134	0	0	0	<0.1
1141	0	2	6	1.8

^a ND, Not done.

^b Data from Pipas et al. (56).

^c Infectivity data from Lai and Nathans (39).

FIG. 3. Replication of cloned SV40 deletion mutant DNA in monkey cells. Subconfluent BSC40 monolayers were transfected with deletion mutant DNA, deletion mutant DNA plus helper *d/1007* DNA, or helper *d/1007* DNA alone as described in the text. At 24 h (lanes a, c, and i), 48 h (lanes b, f, and j), 60 h (lanes c, g, and k), and 72 h (lanes d, h, and l) Hirt DNA was prepared, digested with *DpnI* and *BclI* (or *BamHI* if the *BclI* site was absent), and fractionated by agarose gel electrophoresis. The DNA was transferred to nitrocellulose (76) and hybridized with SV40 [³²P]DNA. Lanes a to d, *d/1007*; lanes e to h, *d/1061*; and lanes i to l, *d/1007* plus *d/1061*.

Viral DNA replication. We next tested whether mutant T antigens retained the viral DNA replication function. These experiments were carried out as described previously (55). In brief, viral DNA was excised from the pBR322 recombinant, cyclized, and applied to BSC40 cells with or without DNA from the T-antigen-producing helper virus *d/1007*. Viral DNA was extracted from infected cells at various times,

digested with *DpnI* and *BclI*, fractionated by agarose gel electrophoresis, and transferred to nitrocellulose filters which were hybridized with SV40[³²P]DNA and exposed to X-ray film. Since *DpnI* sites (GA^mTC) are present in SV40 DNA propagated in Dam⁺ strains of *E. coli*, but not in DNA propagated in BSC40 cells, *DpnI* degrades only the input DNA. *BclI* cleaves the single TGATCA site in newly synthesized form I and form II SV40 DNAs, converting them to full-length linear molecules. An example of this assay is shown in Fig. 3, and the overall results are summarized in Table 3.

As expected, viable mutants *d/1066* and *d/1140* were positive for DNA replication in the absence of a helper, although DNA replication was reduced to about one-third that of wild-type SV40 DNA. All nonviable mutants with deletions within the T-antigen gene were unable to replicate their DNA. Since all of these mutant DNAs (including *d/1139*) replicated in cells coinfecting with *d/1007*, the replication defects were all *trans*-complementable, presumably via the *d/1007* T antigen. (Similar results have been obtained in T-antigen-containing Cos cells [22] supplied by Y. Gluzman.) We conclude that none of the mutant T antigens are active in viral DNA replication, except those with changes confined to the carboxyl-terminal region of the protein.

Of the two mutants with deletions outside the coding sequences for T antigen, *d/1134* failed to

TABLE 3. DNA replication and transformation assays

Mutant no.	DNA replication		Transformation	
	Mutant alone	+ <i>d/1007</i>	Foci per dish ^a	% of wild type
1135	—	+	0	<0.1
1047	—	+	0	<0.2
1151	—	+	0	<0.2
1140	+ ^b	+	70	80
1136	—	+	ND ^c	
1137	—	+	0	<0.2
1138	—	+	0	<0.2
1046	—	+	0	<0.1
1001	—	+	0	<0.2
1055	—	+	0	<0.2
1139	—	+	0	<0.2
1058	—	+	0	<0.2
1061	—	+	0	<0.1
1066	+ ^b	+	65	72
1134	—	—	ND	
1141	+ ^d	+	70	80

^a The values are the average of six dishes.

^b Approximately one-third of the wild-type value.

^c ND, Not done.

^d Approximately equal to the wild-type value.

replicate in the presence or absence of *dl1007*, indicating a *cis*-acting defect, most likely a defective replication origin, whereas *dl1141* replicates without a helper.

Cell transformation. T-antigen mutants were tested for their ability to transform rat embryo fibroblasts by the focus assay (86). Cell monolayers were infected with recombinant plasmids by the calcium phosphate method of Graham and van der Eb (24), and foci were scored at 2 and 3 weeks after infection. Only those mutants that retained the ability to replicate were active in the transformation assay (Table 3).

DISCUSSION

The objective of our studies is to determine whether SV40 T antigen has separate functional domains to account for the various biological and biochemical activities attributed to this protein. The experimental approach that we have taken is to construct mutants in vitro with deletions in the gene for T antigen, analyze the mutant DNAs to define the mutational changes at the nucleotide sequence level, and then test the mutants for the various activities attributed to T antigen. In this paper we report on the construction and analysis of a set of mutants with deletions in various parts of the T-antigen gene and their ability to form plaques, to complement early or late SV40 mutants, to make immunologically detectable T antigen, to replicate their DNA in permissive cells, and to transform nonpermissive cells. In the accompanying paper Soprano et al. (75) report on the ability of the same mutants to stimulate host cell DNA synthesis and to reactivate silent rRNA genes in hybrid cells.

First we comment on the sequence changes in the set of mutants isolated. Most carry simple deletions, but several mutants isolated in monkey cells also have insertions or duplications of short DNA sequences. Such complex changes have been noted earlier among mutants isolated in monkey cells (26). The explanation for such insertions or duplications is still unknown. Of 15 mutants with changes in the T-antigen gene analyzed, 4 contained in-phase deletions, one near the beginning of the T-antigen coding sequence, two near the middle, and one near the end of the sequence. Eleven T-antigen deletions were out of phase, creating termination codons at various points in the gene. These mutants would be expected to produce segments of the T-antigen polypeptide starting at the normal amino terminus and ending a variable distance toward the carboxyl terminus, often with an abnormal stretch of amino acids at the carboxyl end.

The expected T antigens were identified previously by immunoprecipitation in only a few cases (56). In this paper indirect immunofluorescence was used to assay for the presence of T-antigen-related polypeptides in BSC40 cells after DNA infections. All of the in-phase deletions (*dl1135*, *dl1047*, *dl1151*, and *dl1140*) produced stronger nuclear fluorescence than did the out-of-phase deletions. With this latter class of mutants, as the predicted amino-terminal fragment of T antigen decreased below about 40% of the length of T antigen (*dl1001*), there was a marked reduction in the amount of fluorescence produced. An occasional weakly fluorescent nucleus was seen with *dl1138*, but fluorescence was not seen with the others (*dl1046*, *dl1137*, and *dl1136*). These results suggest that an amino-terminal fragment of T antigen of 272 amino acids long is required for stability of the protein or transport to the cell nucleus or both. It should be noted that the putative *dl1137* and *dl1138* T-antigen polypeptides would be expected to have additional amino acids at their carboxyl ends; the effect of these residues on stability or nuclear transport is of course unknown.

The other T-antigen activities that were measured, namely, viability, viral DNA replication, and transformation of rodent cells, varied coordinately among the mutants tested. The only mutants that retained these activities were those with deletions confined to the coding sequence for the carboxyl end of the protein, whether in phase or out of phase (*dl1140* and *dl1066*). Viable mutants with similar properties have been described previously (6, 10, 70). Therefore, our present collection of deletion mutants does not allow us to assign different structural domains of T antigen to the replication and transforming functions of the protein. The results do not argue against the existence of such domains, however, since the gross changes in T-antigen structure anticipated by deletion of coding sequences may affect the function of the remaining parts of the molecule. In fact, Stringer (78), Gluzman (personal communication), and Pearson-White and Nathans (unpublished data) have recently isolated mutants of T antigen which have lost the replication function, but have retained transforming activity. Other, perhaps more restricted biochemical activities, such as the ATPase activity, binding to the origin of replication, or binding to the cellular 53,000- to 55,000-dalton protein, may show differential changes among the various shortened T antigens. In contrast to the failure to dissociate replication and transforming functions of T antigen with the deletion mutants described in this report, it has been possible to dissociate stimulation of host cell DNA synthesis and rRNA synthesis from each other and from the replication and transforming

functions, as described in the following paper (75).

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