Helicase, DNA-Binding, and Immunological Properties of Replication-Defective Simian Virus 40 Mutant T Antigens

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Simian virus 40 T antigen (TAg) exhibits nonspecific and origin-specific DNA binding (*ori* binding) and ATPase and helicase activities, all of which are related to its roles in viral DNA replication. We have characterized some of the properties of four replication-defective but transformation-competent mutant TAGs, C6-2, T22, C11, and C8A. C6-2 and T22 TAGs were each previously determined to lack *ori*-binding properties, while C11 TAG was reported to lack ATPase activity. The C8A TAG did not exhibit defects in either *ori*-binding or ATPase functions. We have analyzed additional aspects of these mutant TAGs pertaining to their helicase, DNA-binding, and immunological properties. With the exception of the C11 TAG, all the other TAGs exhibited helicase activity. The lack of helicase activity by C11 TAG was consistent with its previously shown inability to hydrolyze ATP or to replicate viral DNA. These results therefore show that *ori*-binding and helicase activities are separate functions of TAG. Wild-type and mutant TAGs bound with similar efficiency to either native or denatured calf thymus DNA-cellulose, indicating no marked differences in their nonspecific DNA-binding properties. We also tested the binding of wild-type and mutant TAGs to a monoclonal antibody, PAb 100, that was previously shown to recognize an extremely small class of TAG that may represent a unique conformational form of the protein. Interestingly, while less than 10% of the wild-type, C6-2, C11, and T22 mutant TAGs were recognized by PAb 100, more than 60% of the C8A mutant TAG was bound by this antibody. Therefore, although no defect in biochemical function was observed with the C8A TAG, its deficiency in viral DNA replication may be related to an unusual conformation, as detected by its dramatically increased recognition by PAb 100. These results show that the helicase activity of TAG is not required for its transformation function.

The simian virus 40 (SV40) large tumor antigen (TAg), the product of the viral A gene, is a multifunctional protein with roles in both viral DNA replication and oncogenic cell transformation (for reviews, see references 15 and 46). That these two functions are discrete and separable has been indicated by the existence both of TAG mutants that are replication-defective but competent to transform cells (16, 23, 26, 32, 44) and, reciprocally, of those that can replicate viral DNA but are deficient in transformation (9, 23, 33). The multifunctional nature of TAG is also reflected in its several biochemical properties. TAG binds nonspecifically to both double- and single-stranded DNA (2, 5, 30, 34, 35, 40). It also binds specifically and with high affinity to sites 1 and 2 within the viral regulatory region (*ori*), which contain multiple copies of the consensus pentanucleotide 5'-GA/GGGC-3' (12, 22). In addition, TAG has been shown to bind ATP (4), to be an ATPase (6, 7), and to exhibit helicase activity in a manner that requires ATP hydrolysis (11, 42). All of these activities are most likely related to its function in viral DNA replication. Two of these properties, namely *ori* binding (23, 27, 36) and ATP hydrolysis (8, 27, 42), have each been shown to be absent or deficient in several replication-defective mutants. That many of these mutant TAGs were also shown to be capable of immortalizing (45) and transforming (23, 26) cells suggests that both *ori*-binding and ATPase activities are not essential for these processes.

The study of SV40 TAG has been aided by the development of systems for the purification of substantial quantities of the viral protein. To further clarify its biochemical functions, analysis of purified replication-defective mutant TAGs

can provide additional useful information. A series of non-conditional replication-defective but transformation-competent mutant TAG genes were generated (17), cloned (16, 26), and then inserted into the helper-independent adenovirus vector AdSVRIII (18, 43). Mutant TAGs purified from recombinant virus-infected human 293 cells were shown to be unable to mediate the replication of viral DNA in vitro, while wild-type TAG that had been similarly expressed and purified exhibited levels of replication activity in vitro that were comparable with other sources of TAG (43). Two of these mutants, C62 and T22, exhibited wild-type ATPase activity but were unable to bind specifically to the viral origin (27, 36, 43). Another mutant, C11, was shown to lack ATPase activity but to exhibit *ori*-binding properties similar to those of wild-type TAG (27, 36). The final mutant, C8A, exhibited both wild-type *ori*-binding and ATPase activities, and therefore posed a mystery as to why it cannot replicate viral DNA (27, 43).

Subsequent to the discovery of its DNA-binding and ATPase properties, TAG was shown to be a helicase and, in the presence of single-stranded binding protein and topoisomerase, to unwind viral DNA at the replication origin (11, 42, 50). Because both the helicase and nonspecific DNA-binding properties of TAG are clearly likely to be related to its function in viral DNA replication, we have determined these properties for this group of mutant TAGs. In addition, we have tested the binding of these proteins to a monoclonal antibody, PAb 100, previously shown to recognize an unusual immunological subclass of SV40 TAG (13, 19, 37).

MATERIALS AND METHODS

Materials. Recombinant adenoviruses, derived from AdSVRIII (18, 43) and encoding wild-type or mutant TAGs, were very generously provided by Y. Gluzman. PAb 100 was

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obtained from the American Tissue Culture Collection, Rockville, Md. The 31-nucleotide (nt) oligomer used for helicase assays was a gift from M. Kenny and J. Hurwitz. The M13 sequencing primer was obtained from New England BioLabs, Inc., Beverly, Mass., and radioisotopes used in these studies were purchased from the Radiochemical Centre, Amersham, England. Protein A Sepharose and A15 M Sepharose were bought from Pharmacia, Inc., Piscataway, N.J. Native and denatured calf thymus DNA-cellulose were from P-L Biochemicals, Inc., Milwaukee, Wis.

Purification of TAGs. Wild-type or mutant TAGs were purified from human 293 cells that were infected with recombinant adenoviruses by using immunoaffinity columns containing TAG monoclonal antibody PAb 419 cross-linked to Protein A-Sepharose by published methods (14, 38). The procedure was modified so that the TAGs were eluted from the antibody columns in buffer containing 20 mM Tris hydrochloride, pH 8.5, 0.5 M NaCl, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, and 50% ethylene glycol. The concentrations of TAGs recovered were approximately equivalent as determined by comparison on silver-stained polyacrylamide gels and were estimated to range from 300 to 500 $\mu\text{g/ml}$. When purified wild-type TAG was added to an *in vitro* replication reaction as previously described (24, 29, 43, 48), approximately 98 to 100 pmol of dTMP were incorporated into acid-soluble material in the presence of a plasmid containing the SV40 replication origin region. Levels of incorporation in the absence of TAG or in the presence of plasmid DNA lacking the SV40 origin were 2.0 and 2.5 pmol, respectively.

Helicase assays. The 15-nt sequencing primer or a 31-nt oligomer (5'-CCGAGGAGGCTTTTGGAGGCCTAGGC TTC-3') that contains 29 nt spanning SV40 TAG binding site 1 was ^{32}P end labeled by kinase (25). A 100-ng sample of either oligomer was annealed to 10 μg of single-stranded circular M13mp9SV0 that contained the SV40 L strand spanning nt 5171 to 294 (2) for 5 min at 95°C, for 20 min at 65°C, and for 20 min at 20°C in a buffer containing 100 mM NaCl, 10 mM Tris hydrochloride, pH 7.5, 10 mM MgCl_2 , and 1 mM dithiothreitol. Labeled heteroduplexes were purified from free oligomers on A-15 Sepharose columns. Helicase reactions containing 2 μg of TAG, 15 ng of ^{32}P -labeled heteroduplex, 25 mM Tris hydrochloride (pH 7.8), 5 mM MgCl_2 , 5 mM ATP, and 5 mM dithiothreitol in 15 μl were incubated for 60 min at 37°C. After addition of DNA sample buffer, reaction mixtures were analyzed by electrophoresis in 10% polyacrylamide gels and autoradiography.

DNA-cellulose binding. Human 293 cells were labeled with [^{35}S]methionine (200 $\mu\text{Ci/ml}$) 2 h prior to extraction at 20 h after infection with recombinant adenoviruses expressing wild-type or mutant TAGs, and DNA-binding procedures were performed with minor modifications as previously described (2). All steps were performed at 4°C. Extracts of 4×10^6 cells were dialyzed against NB buffer containing 0.1 M NaCl, 10 mM potassium phosphate, pH 6.2, 1 mM dithiothreitol, 0.5% Nonidet P-40, and 10% glycerol. Dialyzed extracts equivalent to 5×10^5 cells were mixed with 200 mg of native or denatured calf thymus DNA-cellulose or cellulose alone that had been pre-equilibrated with NB buffer. After being rocked at 4°C for 1 h, the mixtures were centrifuged for 2 min in an Eppendorf centrifuge and the supernatants containing nonbound proteins were collected. The pellets were suspended by stirring them in B buffer containing 0.3 M NaCl, 50 mM Tris hydrochloride (pH 8.5), 1 mM dithiothreitol, 0.5% Nonidet P-40, and 10% glycerol for 30 min. After the bound proteins eluted from the cellu-

lose or DNA-cellulose in B buffer were similarly collected, the mixtures were suspended in B+ buffer containing 1.0 M NaCl, and the tightly bound proteins were collected as before. Between each step, mixtures were washed extensively with the corresponding elution buffer to remove all unbound material. Equal quantities of the unbound, bound, and tightly bound fractions were adjusted to 0.3 M NaCl, pH 7.5, and then immunoprecipitated with TAG monoclonal antibody PAb 419-protein A Sepharose beads. TAG was eluted from the beads by boiling the beads in electrophoresis sample buffer and was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography as described previously (2).

Densitometry. To estimate relative quantities of [^{35}S]methionine-labeled TAGs, autoradiograms were scanned with a densitometer (Bio-Rad Laboratories, Richmond, Calif.). Quantification of densitometry was performed by published procedures (31).

RESULTS

Defectiveness of C11 mutant TAG in helicase activity. TAGs expressed from 293 cells infected with recombinant adenoviruses encoding either wild-type or mutant proteins were purified by immunoaffinity procedures. The wild-type TAG purified in this manner and tested in the *in vitro* DNA replication reaction exhibited levels of activity similar to those previously reported (see Materials and Methods). To test the helicase activities of wild-type and mutant TAGs, their ability to displace end-labeled oligonucleotides annealed to complementary single-stranded circular DNA templates was analyzed. Either the 15-nt sequencing primer or a 31-nt oligomer spanning SV40 TAG binding site 1 on the SV40 E strand was hybridized to single-stranded M13mp9SV0 containing SV40 DNA from nt 5174 to 294. The latter hybrid created TAG high-affinity binding site 1 as the double-stranded part of the heteroduplex. Both labeled substrates were incubated with the various purified TAGs in the presence or absence of ATP. Wild-type TAG and mutants C62, T22, and C8A were all capable of displacing substantial quantities of the labeled 15- and 31-nt oligomers (Fig. 1, Table 1). In all of these cases, the helicase activity depended upon the presence of ATP in the reaction, as has been previously shown for wild-type TAG (11, 42). By contrast, the C11 TAG was markedly less efficient in ATP-dependent helicase activity. The relative quantity of oligonucleotides displaced by C11 was at least fivefold lower than that of the other TAGs tested (Table 1) and frequently exhibited no increase over the quantity observed when no TAG was added to the reaction. As the helicase activity of the other TAGs was dependent upon ATP, it is reasonable to assume that at least one of the reasons why C11 cannot function effectively as a helicase is because of its previously determined failure to hydrolyze ATP. It is interesting that the quantities of the 31-mer displaced by wild-type, C6, or T22 TAGs were similar despite the fact that this substrate contained the high-affinity TAG binding site 1 within the duplex region. Both mutants are unable to bind specifically and tightly to this region, in contrast to wild-type TAG. Thus the specific *ori*-binding property of TAG did not affect the efficiency of oligonucleotide displacement under these conditions.

Wild-type and mutant TAGs exhibit similar nonspecific DNA binding. TAG bound to both double- and single-stranded DNA nonspecifically. This property may be necessary for its role in viral DNA replication, especially if, as has been suggested (41, 47, 49), TAG is required for the elongation

FIG. 1. Helicase activity of TAGs. TAGs are indicated at top of autoradiograms. Each (2.5 μ g) was incubated with helicase buffer containing 32 P-labeled 31-mer annealed to M13mp9SVO in the presence (+) or absence (-) of 5 mM ATP, and then analyzed by electrophoresis on 10% polyacrylamide gels and by autoradiography.

stages of DNA synthesis. Furthermore, the sequences contributing to specific and nonspecific DNA binding are located within different regions of the TAG polypeptide (34). One assay that has proven useful for studying nonspecific DNA binding is retention on DNA-cellulose matrices (1). This assay has provided estimates of both the efficiency of TAG binding (i.e., the proportion of total protein bound by DNA) and the ability of the protein to bind tightly to DNA as measured by criteria such as salt sensitivity (2, 5, 30, 35). The binding of the wild-type and mutant TAGs to double- or single-stranded calf thymus DNA-cellulose was compared in order to determine the relative efficiencies of their retention and the proportions that were more tightly bound. Of particular interest was the C8A TAG, which exhibited wild-type *ori*-binding and ATPase functions and comparable levels of helicase activity.

Extracts of [35 S]methionine-labeled 293 cells that had been infected with recombinant adenoviruses encoding either wild-type or mutant TAGs were dialyzed against binding

(NB) buffer and incubated with samples of either single- or double-stranded DNA-cellulose or cellulose alone that had been treated similarly to that which had undergone coupling to DNA (1). The initial binding conditions, i.e., pH 6.2 and 0.1 M NaCl, had previously been determined to result in the most efficient binding of wild-type TAG to DNA-cellulose. The results of a typical experiment, showing the proportions of unbound, bound, and tightly bound wild-type TAG, are shown in Fig. 2. As can be seen, the vast majority of labeled

TABLE 1. Helicase activity of mutant TAGs^a

TAG	5 mM ATP	32 P-labeled oligonucleotide displaced (fmol)
WT	+	134
WT	-	9
T22	+	118
T22	-	21
C62	+	98
C62	-	13
C8A	+	133
C8A	-	34
C11	+	23
C11	-	0

^a TAGs (2.5 μ g) were incubated in helicase buffer containing the 32 P-labeled 15-nt sequencing primer annealed to M13mp8 and analyzed by polyacrylamide gel electrophoresis and autoradiography. Bands corresponding to the displaced oligonucleotide were identified in autoradiograms, excised from dried gels, and counted by liquid scintillation. The amount of labeled oligonucleotide (19 fmol) displaced from the heteroduplex substrate in the absence of TAG was subtracted from these values.

FIG. 2. Nonspecific binding of TAG to DNA-cellulose. Extracts of [35 S]methionine-labeled AdSVRIII-infected 293 cells were dialyzed against DNA cellulose NB buffer and incubated with cellulose (lanes b to d), double-stranded calf thymus DNA-cellulose (lanes e to g), or single-stranded calf thymus DNA-cellulose (lanes h to j). Samples not retained in NB buffer (lanes b, e, and h) or retained and eluted with B buffer (lanes c, f, and i) or not eluted with B buffer but eluted with B+ buffer (lanes d, g, and j) were immunoprecipitated with PAb 419-Sepharose and then analyzed by electrophoresis on 12% polyacrylamide gels and by autoradiography. Lane a, Total immunoprecipitate from 5×10^5 cells. Lane M, 14 C-labeled protein size markers in kilodaltons as indicated on the left.

TABLE 2. Binding of TAGs to double- and single-stranded DNA-cellulose^a

TAG	Binding (% of total eluted) to double-stranded DNA-cellulose			Binding (% of total eluted) to single-stranded DNA-cellulose		
	NB ^b	B ^c	B+ ^d	NB ^b	B ^c	B+ ^d
Wild type	31.2	66.1	2.8	32.6	62.2	5.2
C6-2	44.2	52.5	3.2	31.8	63.1	5.0
T22	35.6	59.1	5.4	41.0	47.5	11.5
C8A	35.4	56.8	7.7	27.4	67.0	5.6
C11	46.5	47.3	6.1	31.2	63.2	5.6

^a TAG polypeptides were quantified by densitometry and normalized by subtracting amounts that bound to cellulose alone in each case. Values are expressed as percentage of total normalized TAG summed from the three DNA fractions.

^b TAG not retained by DNA-cellulose and eluted in buffer containing 0.1 M NaCl, pH 6.2.

^c TAG retained by DNA-cellulose and eluted in buffer containing 0.3 M NaCl, pH 8.5.

^d TAG retained by DNA-cellulose in 0.3 M NaCl, pH 8.5, and eluted in buffer containing 1.0 M NaCl, pH 8.5.

TAG did not bind to cellulose alone at pH 6.2, although a small proportion (<5%) bound and was eluted at 0.3 M NaCl, and a smaller proportion (<0.5%) was eluted by 1.0 M NaCl. By contrast, between 50 and 65% of the TAG bound to either single- or double-stranded DNA-cellulose and was eluted at 0.3 M NaCl, and a further 3 to 10% eluted at 1.0 M NaCl. A 20,000-*M_r* truncated TAG (trTAG), expressed in cells infected with derivatives of the recombinant adenovirus (AdSVRIII) and estimated to contain amino acids 1 to ~130 (29), did not bind to either double- or single-stranded DNA-cellulose. This is consistent with previous studies showing that small t antigen does not bind DNA (35) and that sequences mapping within the COOH-terminal portion of TAG are required for nonspecific DNA binding (34). Densitometry of all the TAG bound and not bound to DNA-cellulose was performed and corrected for the amounts bound to cellulose alone. Under the experimental conditions described, between 50 and 70% of either wild-type or each mutant TAG bound to DNA-cellulose, and the majority was eluted at 0.3 M NaCl in all cases (Table 2). No significant or striking differences between either their binding to single- or double-stranded DNA-cellulose or their binding very tightly to either type of DNA-cellulose was noted. Thus all the mutant TAGs tested, including C8A TAG, exhibited similar nonspecific binding to both single- and double-stranded DNA-cellulose.

The specific DNA-binding properties of these TAGs to viral origin DNA fragments has been characterized by using both immunobinding (27, 28, 36) and filter-binding (43) assays. As described above, C8A and C11 bound specifically to viral DNA regulatory sequences, while C6-2 and T22 were shown to lack origin-specific DNA-binding activity. These results thus extend previous reports (34, 37) that the non-specific and specific DNA-binding properties are two separable activities of SV40 TAGs. Furthermore, the binding of C11 TAG to DNA-cellulose indicated that this property is independent of the ATPase function of TAG.

Binding of wild-type and mutant TAGs to monoclonal antibody PAb 100. Studies on the structure and function of TAG have been facilitated by the generation of several monoclonal antibodies directed against various epitopes on the wild-type protein (3, 19–21). One of these monoclonal antibodies, PAb 100 (19), has exhibited unique properties. It was shown to recognize a small subclass of TAG which consti-

TABLE 3. Proportion of TAG recognized by PAb 100^a

TAG	% of PAb 416-bound TAG recognized by PAb 100
Wild type.....	3.6
C11.....	10.7
T22.....	4.5
C6-2.....	0.0
C8A.....	60.3

^a Extracts of 10⁵ 293 cells infected with ADSVRIII recombinant viruses expressing wild-type or mutant TAGs were incubated with either purified PAb 416 or PAb 100 (at predetermined equivalence levels) and then immune complexes were precipitated with goat anti-mouse immunoglobulin G bound to inactivated *Staphylococcus aureus* bacteria. TAG polypeptides were released from complexes and analyzed by polyacrylamide gel electrophoresis and autoradiography. Bands corresponding to TAGs were quantified by densitometry, and the percentage of PAb 416-bound TAG that was recognized by PAb 100 was estimated.

tuted approximately 10% of the total immunoreactive protein (19, 37). Intriguingly, despite the small proportion of the TAG that was bound by PAb 100, when this antibody was used in the DNA-binding immunoassay, the majority (60 to 80%) of the DNA-binding activity was immunoprecipitated (37). That the antibody may recognize a unique DNA-binding subclass of TAG was further supported by the fact that the C6-2 *ori*-binding-defective TAG was not recognized by PAb 100 (36). Thus, the antibody appeared to recognize a unique DNA-binding subclass of TAG that was absent in the C6-2 TAG population. To continue the analysis of the PAb 100 antibody and to further characterize its epitope, the abilities of the various mutants to bind this antibody were compared with that of another TAG-specific monoclonal antibody, PAb 416, which has been shown previously to recognize the great majority of the immunoreactive TAG extracted from cells (21, 37). TAGs present in [³⁵S]methionine-labeled extracts of wild-type or mutant-infected 293 cells were bound to either antibody, and then autoradiograms of immunoprecipitates were quantitated by densitometry. A small proportion of wild-type, C11, and T22 TAGs was recognized by PAb 100 (Table 3). Consistent with previous studies, C6-2 TAG was not bound to PAb 100. However, the ability of the T22 TAG to bind PAb 100 to an extent similar to that observed with wild-type TAG showed that PAb 100 does not recognize a specific DNA-binding subclass that is absent in all *ori*-binding-defective TAGs.

Surprisingly, in contrast to the other TAGs, there was a dramatic increase in the amount of C8A TAG recognized by PAb 100 such that more than 60% of the total immunoreactive C8A protein was immunoprecipitated by this antibody (Table 3 and Fig. 3A). Furthermore, in contrast to the wild-type (Fig. 3A) or other mutant (data not shown) TAGs, the 20,000 *M_r* trTAG encoded by C8A was immunoprecipitated with PAb 100. As the trTAG contains neither the PAb 100 epitope (13) nor the C8A mutation as mapped by marker rescue (26), it is conceivable that this fragment associates with the C8A TAG but not with the wild-type TAG. The increased binding of the C8A TAG to PAb 100 compared with that of wild-type TAG was observed not only with metabolically labeled TAG in extracts of infected 293 cells but also in immunoaffinity-purified preparations of TAG (Fig. 3, right) as detected by silver staining. The absence of the trTAG in the preparations of purified wild-type or C8A TAGs is most likely due to the fact that this polypeptide is removed very inefficiently from Pab 419-protein A Sepharose columns with the

FIG. 3. Binding of wild-type and C8A TAGs to PAb 100. (A) Extracts of 10^5 [35 S]methionine-labeled 293 cells infected with Ad5SVR111 recombinant viruses expressing either wild-type (lanes a to c) or C8A (lanes d to f) TAGs were immunoprecipitated with either PAb F4 (anti-polyoma TAG) (lanes a and d), PAB 416 (lanes b and e), or PAb 100 (lanes c and f) and then autoradiographed. Arrows indicate TAG and trTAG. Positions of 14 C-labeled size markers (in kilodaltons) are indicated on the left. (B) A 1- μ g sample of either purified wild-type (lanes g to i) or C8A (lanes j to l) TAG was immunoprecipitated with PAb F4 (lanes g and j), PAB 416 (lanes h and k), or PAb 100 (lanes i and l) followed by polyacrylamide gel electrophoresis and silver staining.

elution buffer employed in our procedures (K. Auborn and C. Prives, unpublished observations).

DISCUSSION

The two most well-studied consequences of the function of SV40 TAG are the replication of the viral genome and the oncogenic transformation of the host cell. Two experimental approaches have provided insight into these processes. First, the generation of large numbers of TAG mutants has provided information about the biological effects and functions of TAG. Second, the development of a number of systems that yield sufficient quantities of TAG for biochemical analysis has led to considerable progress in determining its biochemical activities during DNA replication. The experiments described herein have combined both approaches. Table 4 provides a summary of the properties of the wild-type and mutant TAGs used in this study. Several points can be made from this compendium.

First, a mutant that lacked ATPase activity also lacked helicase activity. Second, neither specific *ori*-binding, ATPase, nor helicase activities of TAG are obligatorily linked to its nonspecific DNA binding properties because under the

conditions tested, all of the TAGs bound to double- and single-stranded calf-thymus DNA-cellulose similarly. Third, as all of these mutants have been previously shown to be capable of transforming both established and primary cells (16, 26), it can be concluded that neither *ori*-binding, ATPase, nor helicase activities are required for the ability of SV40 TAG to alter the growth properties of cells. Presumably, one or more of the biochemical properties of TAG that are related to its transformation function(s) remain to be identified.

The binding of PAb 100 to wild-type and mutant TAGs was informative for two reasons. First, it demonstrated that not all *ori*-binding-defective TAGs lack the PAb 100 epitope. This antibody was previously shown to recognize less than 10% of the TAG from Cos cells, but in the DNA-binding immunoassay it immunoprecipitated 60 to 80% of its *ori*-binding activity (37). The fact that the C6-2 mutant TAG which fails to bind specifically to the *ori* is also not recognized by PAb 100 supported the suggestion that PAb 100 recognizes a DNA-binding subclass of TAG (36). The results of the current study contradict our previous conclusion, because the T22 TAG that also lacks *ori*-binding activity is recognized by this antibody to the same extent as wild-type TAG. The PAb 100-specific conformation, if necessary at all, is clearly not sufficient for specific *ori* binding.

The second reason that the PAb 100 study was useful was that it revealed a possible clue as to why the C8A TAG is unable to replicate viral DNA. Among the mutants tested, only the C8A TAG exhibited no defects either in specific or nonspecific DNA binding or in ATPase and helicase activities. This may suggest that there is another biochemical function of TAG that is important for viral replication, such as binding to DNA polymerase (39), or that there is some additional TAG property that is required for unwinding DNA at the origin. However, although a specific biochemical defect of C8A TAG has not yet been reported, we have observed that this mutant differs immunologically from the other proteins tested. Monoclonal antibody PAb 100 was shown to bind to a markedly higher proportion of C8A TAG than the other TAGs that were tested. Studies on the interactions of PAb 100 with TAG have indicated that it recognizes a small subclass of TAG that is determined by the conformation of the protein. Depletion of all of the PAb 100-bound TAG population required repeated rounds of immunoprecipitation even in the presence of excess antibody, suggesting that this represents a dynamic subpopulation (19; A. Scheller and C. Prives, unpublished). While the precise mapping of its epitope has not been reported, PAb 100 recognizes the SV40 truncated polypeptides encoded by the adenovirus-SV40 hybrid virus, Ad2⁺ND₂, that are encoded by sequences between 0.43 and 0.28 map units on the viral genome (approximately amino acids 300 to 525) (13). Its epitope therefore lies outside of the region containing the C8A mutation that is responsible for its inability to replicate viral DNA. The proportion of the Ad⁺ND₂ 42-kilodalton truncated TAG that was recognized by PAb 100 was markedly greater than that of full-length TAG, suggesting that the conformation of full-length wild-type TAG reduces accessibility of the epitope to this antibody (13). Further support for a PAb 100 conformation-dependent epitope is derived from our observations that TAG binding to PAb 100 is denaturation sensitive and increases upon mild heat treatment of the viral antigen (10). That PAb 100 recognized 6- to 10-fold greater quantities of C8A TAG than it did of wild-type, T22, or C11 TAGs supports the possibility that the C8A mutant TAG has an altered conformation. This alteration, which was

TABLE 4. Properties of mutant TAGs

TAG	Mutation	<i>ori</i> -Binding	ATPase activity	Helicase activity	Binding to:	
					PAb 100	DNA-cellulose
C6-2	Asn-153→Thn	—	+	+	—	+
T22	His-203→Gln	—	+	+	+	+
C8A	Lys-224→Glu	+	+	+	+++ ^a	+
C11	Pro-522→Ser	+	—	—	+	+

^a PAb 100 binding to C8A TAG was 6- to 10-fold higher than it was to the other TAGs.

detected by antibody recognition, may be responsible for the inability of this mutant to replicate viral DNA.

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