JOURNAL OF VIROLOGY, Oct. 1985, p. 102–109
0022-538X/85/100102-08$02.00/0

Vol. 56, No. 1

Origin Binding by a 100,000-Dalton Super-T Antigen from SVT2 Cells

VAN G. WILSON* AND RUBY L. WILLIAMS

Department of Medical Microbiology and Immunology, College of Medicine, Texas A&M University, College Station, Texas 77843

Received 15 February 1985/Accepted 19 June 1985

The SVT2 line of simian virus 40-transformed mouse cells expresses little or no wild-type-size A protein (T antigen). Instead, a variant form is produced in these cells that is larger than normal-size A protein. This variant form has an M_r of 100,000 (100K super-T antigen) and is found primarily in complexes with the host-cell-coded p53 protein. Binding of the 100K super-T antigen to simian virus 40 origin region DNA was assayed by immunoprecipitation of super-T antigen–DNA complexes and then digestion with DNase I. DNA sequences associated with super-T antigen were protected from digestion and retained in the immune complex, while unprotected sequences were digested and released. The 100K super-T antigen efficiently protects DNA sequences in the previously defined regions I and II (P. Tegtmeyer, B. A. Lewton, A. L. DeLucia, V. G. Wilson, and K. Ryder, J. Virol. 46:151–161, 1983). Within region II (the origin of replication), the pattern and size of protected fragments are identical for super-T antigen and purified wild-type A protein. Thus, even though super-T antigen is larger than wild-type A protein, both must bind with the same alignment on origin DNA. Furthermore, complexes between the host-cell-coded p53 protein and the 100K super-T antigen also retain the ability to bind in regions I and II.

Simian virus 40 (SV40) A protein (large T antigen) is required for the initiation of SV40 DNA replication (26). Binding and mutational data suggest that a direct interaction of A protein with sequences at the viral origin of replication is necessary for this replicative function (8, 9, 18, 20, 27). In vitro, purified SV40 A protein specifically binds to multiple sites within three contiguous regions (I, II, and III) on the SV40 genome (22). Bound A protein protects a minimum of 30 to 35 base pairs of DNA from digestion with DNase I (6, 11, 22, 24, 25, 28). In addition, sequential binding by A protein to the multiple sites in region II results in the protection of four size classes of DNA fragments ranging from 30 to 35 up to 60 to 65 base pairs in length (22). The boundaries of the protected fragments derived from both regions I and II have been mapped previously and are summarized in Fig. 1. Since the bondaries of binding region II correspond extremely well with the boundaries of the functional origin of replication (8, 22), it is likely that a precise adherence to specific interactions with region II sequences is required for A protein to perform its function in replication (6).

Altered-molecular-weight forms of A protein are often observed in transformed cells, and the forms that are larger than wild-type A protein are referred to collectively as super-T antigens (2, 5, 13–15). Super-T antigens appear to arise from internal duplications within the coding region of the A gene (13, 14). What effect these structural changes have on the various functional properties of A protein is currently under investigation (2, 4, 5, 15). It has been shown recently that super-T antigens from both rat and mouse transformed cell lines are defective for replicative function, yet they retain DNA-binding activity (2, 15). In each case, the super-T antigens preferentially bound to restriction fragments containing the SV40 origin region. These assays are limited, however, in that they do not provide information about the pattern of binding or the alignment of super-T antigens on the multiple sites within regions I and II. It is conceivable that the super-T antigens may bind to the origin, but in an altered fashion that is incompatible with the replicative function. For the present study, an assay was developed which allows specific binding in regions I and II to be determined with whole-cell extracts as a source of A protein. The interaction between a 100,000-M_r (100K) super-T antigen from SVT2 cells and SV40 regions I and II was examined by this assay.

MATERIALS AND METHODS

Cells. CV-1 cells obtained from P. Tegtmeyer were maintained in Dulbecco modified Eagle medium with 5% fetal calf serum. COS cells obtained from J. Nordstrom and SVT2 cell obtained from the American Type Culture Collection were maintained in Dulbecco modified Eagle medium with 10% fetal calf serum. Productive infection with SV40 strain VA 45-54 was as described by Tegtmeyer and Andersen (21).

Antisera and monoclonal antibodies. Pooled hamster antitumor serum (HAT) and normal hamster serum (NHS) were described previously (21). The mouse hybridoma lines PAb101 and PAb122 were obtained from the American Type Culture Collection. The cell culture media of these hybridomas were used as a source of monoclonal antibodies specific for SV40 A protein and the host-cell-coded p53 protein, respectively. All immunoprecipitations were performed with a single batch of pooled culture supernatants from the appropriate hybridoma culture.

Protein purification. SV40 A protein was purified from productively infected CV-1 cells by the method of Tegtmeyer and Andersen (21).

Preparation of DNA. SV40 DNA was purified, nick translated, and when necessary, digested with restriction enzymes as previously described (22).

Radiolabeling and extraction of cells. Forty-eight hours after infection, 150-cm^2 flasks of CV-1 cells were incubated for 1 h in 10 ml of methionine-free minimal essential medium supplemented with 5% dialyzed fetal calf serum. After 1 h,

* Corresponding author.

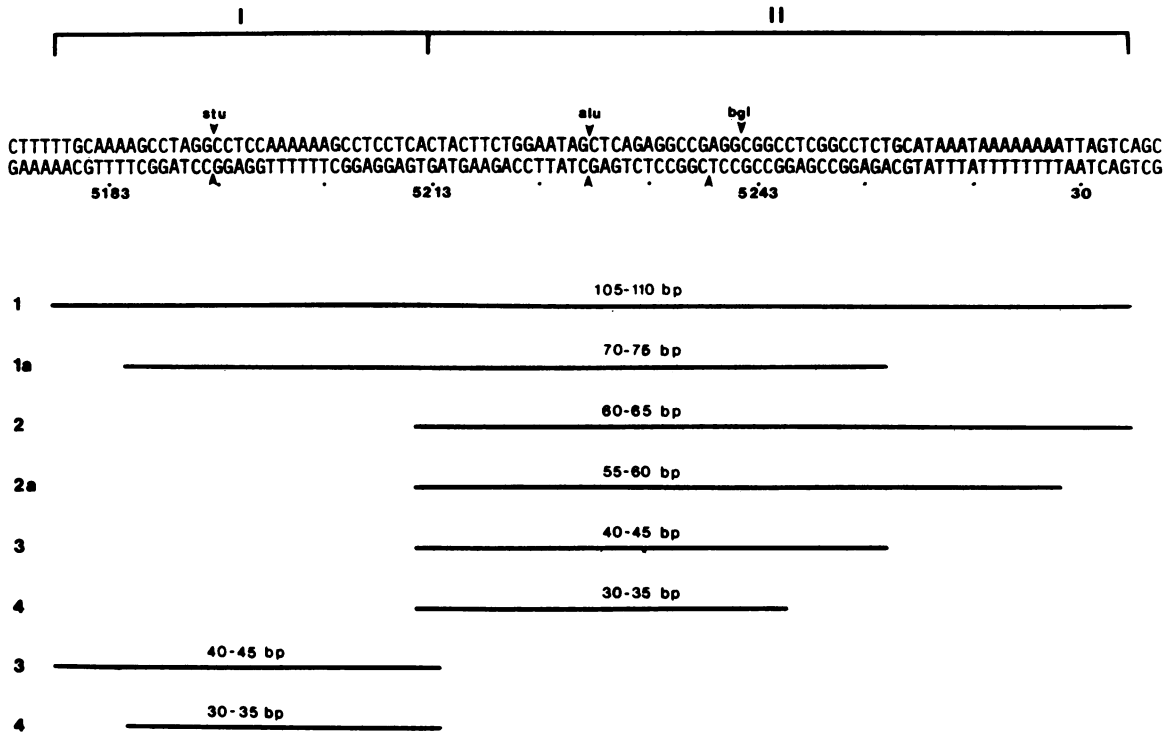

FIG. 1. Summary of DNase-protected fragments mapped to regions I and II. The DNA sequence from the SV40 origin region is shown with nucleotide numbering by the system of Buchman et al. (described in reference 26). The cleavage sites for restriction enzymes mentioned in the text are indicated. Above the sequence are the overall boundaries of regions I and II as determined by DNase footprinting (22). Below the sequence are the locations of the previously mapped protected fragment. Fragments 1 and 1a span both regions, fragments 2 and 2a are specific for region II, and fragments 3 and 4 can be generated by A protein bound to either region I or II.

the methionine-free medium was removed, and cells were labeled for 3 h by the addition of 50 μCi of L-[^{35}S]methionine (1,058 Ci/mmol; New England Nuclear Corp.) in 10 ml of fresh methionine-free medium. Freshly confluent monolayers of COS or SVT2 cells were labeled by the same protocol.

Labeled and unlabeled cell cultures were extracted in the same fashion. After removal of the medium, cells were scraped in 10 ml of ice-cold phosphate-buffered saline, washed twice with ice-cold phosphate-buffered saline, and suspended at 5×10^7 cells per ml in 20 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), pH 8]–150 mM NaCl–0.1 mM EDTA–1 mM dithiothreitol–0.5% Nonidet P-40–0.25% (wt/vol) phenylmethylsulfonyl fluoride. After 1 h at 4°C, the suspension was centrifuged for 5 min at 12,000 × *g*. The supernatant was brought to pH 7 with acetic acid, made 10% with glycerol, and stored at −70°C.

Immunoprecipitation and electrophoresis of labeled proteins. Labeled extracts (15 to 50 μl) were incubated with 10 μl of HAT or NHS or with 250 μl of hybridoma culture supernatants. Final volumes were adjusted to 300 μl in each case with phosphate-buffered saline. After 60 min at 4°C, 50 μl of a 10% (wt/vol) suspension of Formalin-fixed *Staphylococcus aureus* cells (Cowan I strain) was added. Incubation was continued for 60 min at 4°C, and the immune complexes were collected by centrifugation at 12,000 × *g* for 2 min. The *S. aureus* pellet was washed twice with 0.5 ml of 20 mM PIPES (pH 7)–75 mM NaCl–10 mM EDTA–0.25% Nonidet P-40–0.1% sodium dodecyl sulfate–0.5% (wt/vol) bovine serum albumin and once with 0.5 ml of 20 mM PIPES (pH 7)–1 mM NaCl–1 mM EDTA. Precipitated proteins were extracted with 40 μl of 75 mM Tris sulfate (pH 8.3)–15% glycerol–2% sodium dodecyl sulfate–1% mercaptoethanol–0.01% bromophenol blue. Samples were incubated for 5 min at 100°C before electrophoresis on 15 or 20% acrylamide gels as described by Tegtmeyer et al. (23).

DNase fragment assay. Complexes between purified A protein and nick-translated SV40 DNA were digested with DNase I, and the protected fragments were collected on nitrocellulose filters as described by DeLucia et al. (7).

Immunoprecipitation fragment assay. Purified A protein (approximately 0.01 μg) or whole-cell extracts (15 to 25 μl) were incubated with nick-translated SV40 DNA in a final volume of 50 μl containing 20 mM PIPES (pH 7), 75 mM NaCl, 0.1 mM EDTA, and 3 to 5% glycerol. After 1 h at 4°C, 50 μl of a 10% (wt/vol) suspension of Formalin-fixed *S. aureus* cells, preloaded with the appropriate antibody (see below), was added, and the incubation was continued for 90 min at 4°C. The immune complexes were precipitated by centrifugation at 12,000 × *g* for 2 min at 4°C. The pellets were washed once with 0.5 ml of ice-cold 20 mM PIPES (pH 7)–75 mM NaCl–0.1 mM EDTA and then resuspended in 50 μl of the same buffer. DNase I (20 μg; Sigma Chemical Co.) in 5 μl of 20 mM PIPES (pH 7)–1 mM NaCl–0.1 mM EDTA–0.5 M $MgCl_2$–50 mM $CaCl_2$ was added for 5 min at 4°C to release nonprotected DNA sequences from the immune complex. DNase digestion was stopped by the addition of 500 μl of ice-cold 20 mM PIPES (pH 7)–75 mM NaCl–10 mM EDTA followed by centrifugation at 12,000 × *g* for 2 min. The *S. aureus* pellet was washed twice with 0.5 ml of 20 mM PIPES (pH 7)–75 mM NaCl–10 mM EDTA–0.25% Nonidet P-40–0.05% (wt/vol) bovine serum albumin–0.01% (wt/vol) sheared herring sperm DNA and once with 0.5 ml of 20 mM PIPES (pH 7)–1 mM NaCl–1 mM EDTA and then suspended in 40 μl of 10 mM Tris borate (pH

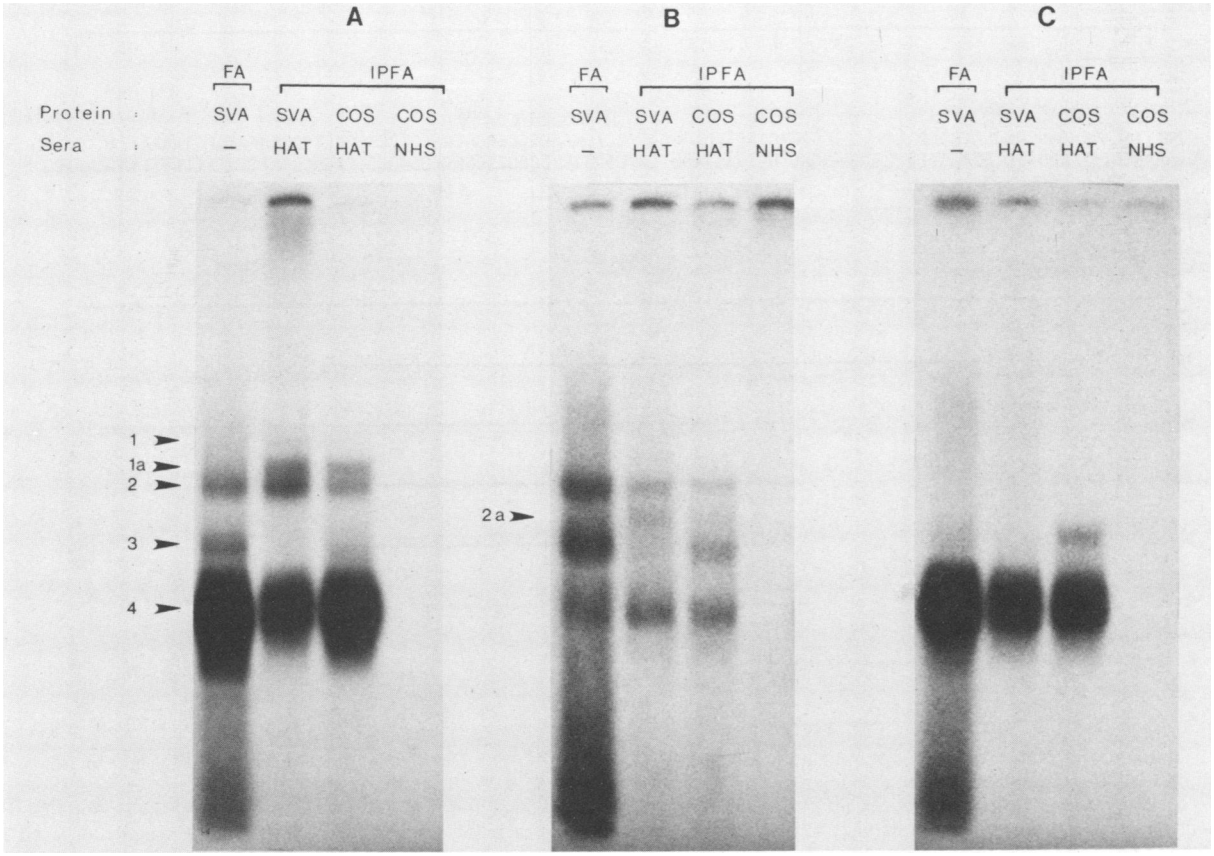

FIG. 2. Comparison of protected fragments recovered by the fragment assay and the immunoprecipitation fragment assay. Protected fragments were recovered either by retention on nitrocellulose filters (FA lanes) or by immunoprecipitation (IPFA lanes) as described in Materials and Methods. The source of A protein was either a purified preparation (SVA lanes) or a whole-cell extract from COS cells (COS lanes). Immunoprecipitation was by HAT or NHS. The SV40 DNA was either uncut (A), precut with *Stu*I (B), or precut with *Alu*I plus *Bgl*I (C). Protected fragments are designated as previously defined (22).

8.3)–0.2 mM EDTA–5% glycerol–0.2% sodium dodecyl sulfate–0.025% bromophenol blue–0.025% xylene cyanol. After 30 min at 25°C, the samples were centrifuged for 2 min at 12,000 × *g*, and the supernatants were loaded onto 12% nondenaturing gels as described by Chandler and Gralla (1). A similar immunoassay for A protein-DNA binding has been described previously by R. McKay (16).

Preparation of *S. aureus*. *S. aureus* cells were Formalin fixed and suspended to 10% (wt/vol) as described by Kessler (12). For the immunoprecipitation fragment assay, *S. aureus* cells were preloaded with antibody by incubating 50 μl of the 10% suspension of fixed *S. aureus* cells with the desired antibody in a final volume of 300 μl. After 60 min at 4°C, *S. aureus* cells were collected by centrifugation for 2 min at 12,000 × *g*. Pellets were washed once with 0.5 ml of 20 mM PIPES (pH 7)–75 mM NaCl–10 mM EDTA–0.25% Nonidet P-40–0.05% (wt/vol) bovine serum albumin–0.01% (wt/vol) sheared herring sperm DNA and resuspended in 50 μl of the same buffer.

RESULTS

Comparison of protected fragments collected by filtration or immunoprecipitation. Purified A protein binds to SV40 DNA and protects specific sequences from digestion with excess DNase I (11, 22, 27). Typically, the DNase-protected fragments are recovered by retention of the protein-DNA complexes on nitrocellulose filters. As an alternative approach to fragment recovery, A protein-DNA complexes were immunoprecipitated first and then DNase digested (Fig. 2). Only DNA sequences bound and protected by A protein should be retained in the immune complex under these conditions. Immunoprecipitation with HAT yielded protected fragments identical in size to the filter-collected fragments 1, 1a, 2, and 4, while little or no fragment 3 was recovered (Fig. 2A, lanes 1 and 2). Recovery of fragment class 3 with HAT was inconsistent from sample to sample, though in general recovery was poor when uncut SV40 DNA served as the binding substrate for A protein. The basis for this variablity in recovery of fragment class 3 when HAT is used for the precipitation is not clear. However, with five A protein-specific monoclonal antibodies (PAb101, PAb402, PAb405, PAb419, and PAb430), all fragment classes including class 3 were recovered consistently in amounts comparable to those recovered by filtration (V. Wilson, unpublished observations). The successful recovery of all fragment classes with the monoclonal antibodies suggests that similar binding events are detected with either the fragment assay or the immunoprecipitation fragment assay.

While immunoprecipitation yielded protected fragments that were equivalent in size to those obtained by filtration, it was necessary to determine whether or not each immunoprecipitated fragment contained the same protected sequences as the corresponding filter-collected fragment. The genomic origins of the fragments collected by filtration

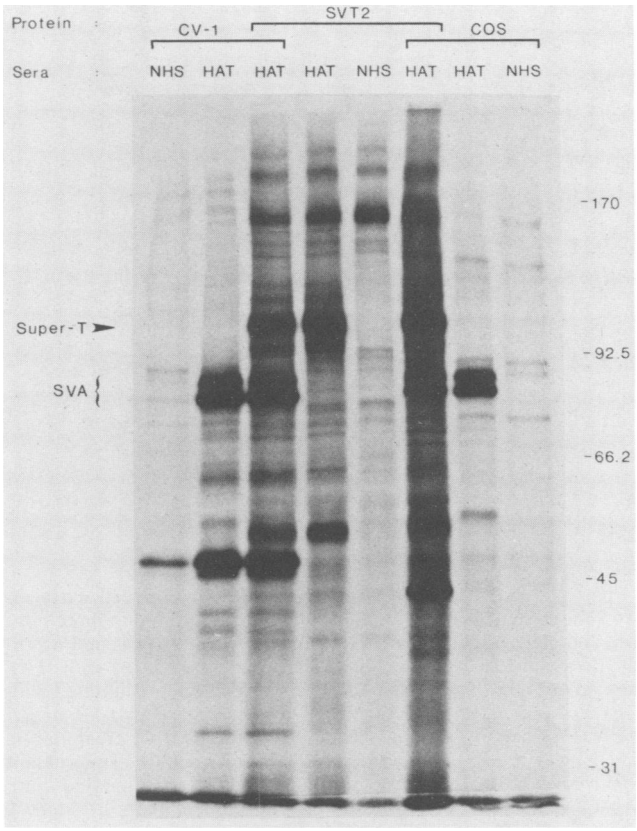

FIG. 3. Comparison of A proteins from COS, SVT2, and SV40-infected CV-1 cells. Extracts from L-[^{35}S]methionine-labeled SVT2, COS, or SV40-infected CV-1 cells were prepared, immunoprecipitated, and electrophoresed as described in Materials and Methods. Immunoprecipitations were with HAT or NHS, as indicated above each lane. Equal trichloroacetic acid-precipitable counts were used for each sample. The positions of the super-T antigen and wild-type A protein (SVA) are indicated. The positions of unlabeled molecular weight markers run on the same gel are indicated on the right.

have been mapped previously (Fig. 1). Cutting SV40 DNA with appropriate restriction enzymes prior to A protein binding produces a characteristic effect on the pattern and size of the resulting protected fragments (22). For example, digestion with *Stu*I eliminates fragment classes 3 and 4, which arise from binding in region I, and truncates fragments 1 and 1a, but does not affect classes 2 through 4, which result from binding in region II (22). Alternatively, precutting SV40 DNA with *Alu*I plus *Bgl*I eliminates those fragment classes generated by A protein bound to region II, but does not affect fragments 3 and 4, which are derived from binding in region I. If the fragments collected by immunoprecipitation and filtration map to the same genomic locations on uncut SV40 DNA, then both methods should give identical protected fragments on precut DNA as well.

On the *Stu*I-cut DNA, immunoprecipitation yielded protected fragments identical in size to fragments 2 and 4 obtained by filtration (Fig. 2B). As on uncut DNA, recovery of fragment 3 with HAT was poor in this experiment. Protected fragment 2a observed in the immunoprecipitated sample also can be obtained by filtration, but for this fragment, recovery seems to be enhanced by immunoprecipitation. Fragments 1 and 1a were absent in both cases, and on longer exposures, a characteristic 85- to 90-base-pair truncated form of fragment 1 could be observed (see Fig. 4, lane 1). After DNase digestion of A protein bound to the *Alu*I-plus-*Bgl*I-cut SV40 DNA, only protected fragment 4 was recovered by either method. In neither case were the fragments generated by binding of A protein to region II sequences (1, 1a, 2, and 2a) present. This correspondence of fragment pattern and size on uncut and two different precut DNAs confirms that the immunoprecipitated fragments map to the same genomic sites as those obtained with the previously employed filtration method.

Recovery of protected fragments by filtration requires a relatively pure source of A protein, while immunoprecipitation of A protein-DNA complexes can be performed with crude extracts (Fig. 2A). SV40-transformed COS cells were used as a source of wild-type A protein. Whole-cell extracts of COS cells were incubated with SV40 DNA, immunoprecipitated, and DNase digested as described in Materials and Methods. Immunoprecipitation with HAT resulted in a fragment pattern equivalent to that seen with purified A protein, while no protected fragments were recovered when NHS was used for the precipitation. Identical results were obtained with extracts from lytically infected CV-1 cells as a source of A protein (unpublished observations). When COS extracts were incubated with precut SV40 DNA, the pattern of DNase-protected fragments was essentially identical to that produced by immunoprecipitation of purified A protein (Fig. 2B and C). In multiple experiments, the patterns differed only in the variability of recovery of fragment class 3. Since the pattern of protected fragments is the same for whole-cell extracts and purified A protein on both uncut and precut DNAs, then the corresponding fragments must originate from the same genomic locations. This finding demonstrates that immunoprecipitation is suitable for examining the binding of unpurified A protein to regions I and II on the SV40 genome.

DNA-binding properties of a super-T antigen. The SVT2 line of SV40-transformed mouse cells has been reported to contain a form of A protein larger than wild type (3, 10, 19). We have confirmed this observation (Fig. 3). Immunoprecipitation of SVT2 cell extracts with HAT yielded a prominent A protein species larger than that found in SV40-infected CV-1 cells or SV40-transformed COS cells. The SVT2 super-T antigen has an apparent molecular weight of 100,000. Little if any wild-type-size A protein was detectable in the SVT2 extracts even upon long exposure such as that shown in Fig. 3 and with a variety of labeling conditions (unpublished observations). This molecular weight difference between A proteins from infected CV-1 cells and from SVT2 cells is not the result of proteolytic processing differences. Mixture of CV-1 or COS extracts with SVT2 extracts prior to immunoprecipitation (Fig. 3, lanes 3 and 6) or coextraction of infected CV-1 cells with SVT2 cells (unpublished observations) had no effect on the observed molecular weight of the SVT2 super-T antigen.

DNA binding by SVT2 super-T antigen in whole-cell extracts was compared with binding by purified wild-type A protein by the immunoprecipitation fragment assay (Fig. 4). On uncut SV40 DNA, the SVT2 extract protected fragments equivalent in size to fragments 1, 1a, 2, and 4 seen with purified A protein. Precutting the DNA with *Stu*I eliminated fragments 1 and 1a from both the A protein and SVT2 samples and resulted in the appearance of a new 85- to 90-base-pair fragment in each case. In addition, super-T antigen protected fragments identical in size to the known region II fragments (2, 2a, 3, and 4) protected by purified A protein bound to *Stu*I-cut SV40 DNA (compare lanes 1 and

2 in Fig. 4). When region II was precut with *Alu*I plus *Bgl*I, both wild-type A protein and SVT2 super-T antigen were able to protect only a 35- to 40-base-pair fragment. Since no differences were observed in the size or number of fragments protected by super-T antigen and purified A protein, then super-T antigen must bind in regions I and II with the same alignment as wild-type A protein.

DNA binding by p53 protein–super-T antigen complexes. Several super-T antigens, including that of SVT2, have been shown to bind to the host cell-coded p53 protein in transformed cells (2, 15, 19). The extent to which super-T antigen and p53 are complexed in SVT2 extracts was addressed by sequential immunoprecipitation of cultures labeled for 3 h with [^{35}S]methionine (Fig. 5). Super-T antigen was cleared from the SVT2 extract by precipitation with an excess of an A protein-specific monoclonal antibody, PAb101. The amount of uncomplexed p53 was determined by immunoprecipitation of the PAb101 supernatant with a p53-specific monoclonal antibody, PAb122 (Fig. 5, lane F). No free p53 was detected by this procedure. The presence or absence of free super-T antigen was determined by first removing p53 with PAb122 and then immunoprecipitating the PAb122 supernatant with PAb101 (Fig. 5, lane H). Under these conditions, a small amount of free super-T antigen was observed. Thus on a long labeling, most if not all the p53

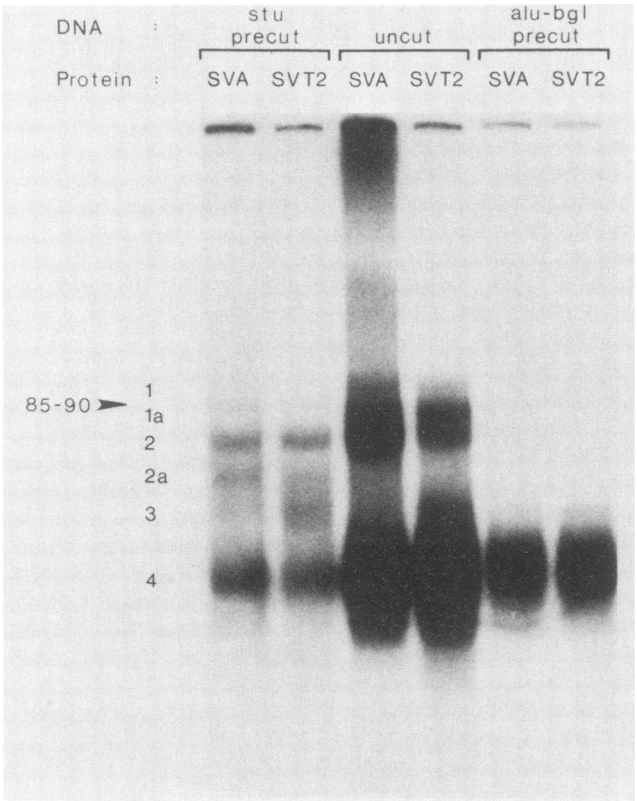

FIG. 4. DNase protection by wild-type A protein and SVT2 super-T antigen. The binding activity of A protein purified from infected CV-1 cells (SVA lanes) and super-T antigen in whole-cell extracts of SVT2 cells (SVT2 lanes) was determined by the immunoprecipitation fragment assay. Binding was assayed with uncut, *Stu*I-precut, or *Alu*I-plus-*Bgl*I-precut SV40 DNA as indicated above the lanes. The position of an 85- to 90-base-pair fragment observed after binding to the *Stu*I-precut DNA is indicated.

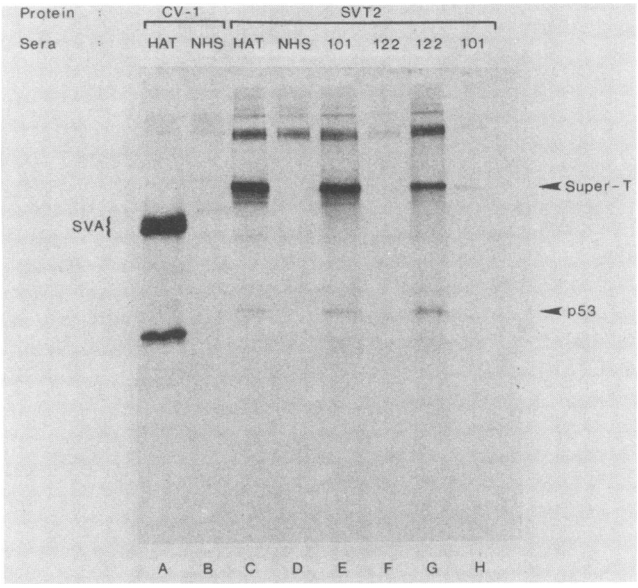

FIG. 5. Sequential immunoprecipitation of SVT2 cell extracts. L-[^{35}S]methionine labeling, whole-cell extraction, immunoprecipitation, and electrophoresis were as described in Materials and Methods. SV40-infected CV-1 cell extracts were precipitated with HAT (lane A) or NHS (lane B). SVT2 cell extracts were precipitated with an excess amount of HAT (lane C), NHS (lane D), PAb101 (lane E), or PAb122 (lane G). The supernatant from the PAb101 precipitation (lane E) was precipitated with PAb122 (lane F), and the supernatant from the PAb122 precipitation (lane G) was precipitated with PAb101 (lane H). The positions of wild-type A protein, super-T antigen, and p53 are as indicated.

seems to be associated with super-T antigen, while at least some super-T antigen remains free of p53 protein.

Previously, SVT2 super-T antigen–p53 protein complexes have been shown to bind specifically to an SV40 origin containing restriction fragment, while free p53 had no affinity for SV40 origin DNA (19). However, this assay did not allow a qualitative analysis of the multiple binding interactions possible within regions I and II. To determine whether or not p53 affected the sites of binding or the alignment of super-T antigen, the immunoprecipitation fragment assay was performed with a p53-specific monoclonal antibody, PAb122 (Fig. 6). On uncut SV40 DNA, immunoprecipitation with PAb122 resulted in protected fragments that were qualitatively identical but quantitatively much less than the fragments recovered by HAT or the A protein-specific monoclonal antibody PAb101. Identical results were obtained with the anti-p53 monoclonal antibody PAb421 (data not shown). No recovery of any protected fragments was detected when the immunoprecipitation was performed with a mock monoclonal antibody.

The low recovery of protected fragments with either PAb122 or PAb421 made examination of the fragment pattern on precut DNA difficult. Nonetheless, faint bands are observed in Fig. 6 at the positions of fragment classes 2 and 3 when PAb122 was used to precipitate SVT2 extracts bound to *Stu*I-precut DNA. Recovery of fragment class 4 from the *Alu*I-plus-*Bgl*I-precut DNA was readily apparent with PAb122. This recovery of DNase-protected fragments when PAb122 was used for the immunoprecipitation step indicates that p53 protein–super-T antigen complexes retain DNA-binding capability. In addition, the sizes and patterns of fragments recovered with PAb122 from uncut and precut

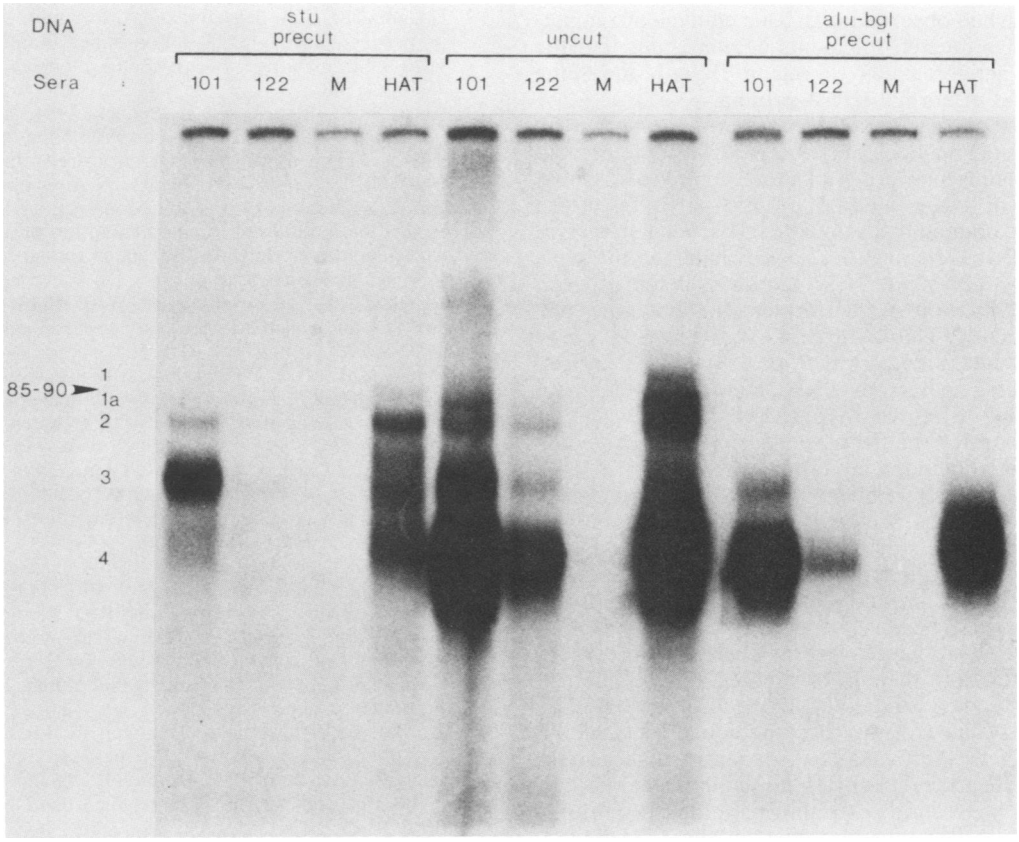

FIG. 6. Analysis of protected fragments recovered with monoclonal antibodies. Whole-cell extracts of SVT2 cells were incubated with *Stu*I-cut, uncut, or *Alu*I-plus-*Bgl*I-cut SV40 DNA as indicated. The immunoprecipitation fragment assay was performed as described in Materials and Methods with PAb101, PAb122, HAT, or a mock monoclonal supernatant derived from a nonproducing hybridoma culture (M lanes).

DNAs are consistent with the p53–super-T antigen complexes binding to and protecting the same SV40 sequences in regions I and II as does purified A protein.

DISCUSSION

DNase digestion of immunoprecipitated A protein-DNA complexes allows the recovery of protected DNA fragments that are identical in size and genomic origin to those collected by filtration. Thus, the immunoprecipitation fragment assay is capable of detecting the multiple binding interactions in regions I and II that have been demonstrated by the previously employed fragment assay (7, 22, 27). As with filtration, precutting the DNA allows one to separate regions I and II so that binding events in either region can be examined independently. Unlike filtration, immunoprecipitation does not require a purified source of A protein. The pattern and alignment of binding by A protein within regions I and II now can be determined with whole-cell extracts as a source of A protein. Consequently, the immunoprecipitation fragment assay should be particularly useful for examining DNA binding by mutant or variant forms of A protein.

The SVT2 line of SV40-transformed mouse cells expresses a 100K super-T antigen while expressing little or no normal-size A protein. The ability of this 100K super-T antigen to function in the initiation of SV40 replication is not yet known; nonetheless, extracts from SVT2 cells efficiently protect SV40 DNA sequences from DNase I digestion. When total amounts of A protein were compared by a quantitative enzyme-linked immunosorbent assay, it was found that equivalent amounts of A protein from SVT2, COS, and SV40-infected CV-1 cells were required to produce equivalent patterns of DNase I-protected fragments (V. Wilson, unpublished observations). Thus it is unlikely that the protection observed with SVT2 extracts is due to any minor fraction of normal-size A protein that might be present in these extracts. Instead, it appears that the 100K super-T antigen retains specific DNA-binding ability.

The ability of the 100K super-T antigen to bind in region II is of particular interest, since the boundaries of protected region II correspond extremely well with the functional origin of replication (8, 22). It has been postulated that the precise arrangement of A protein protomers on the four identical pentanucleotide sequences in region II may be crucial for the functional role of A protein in the initiation of replication (6, 22). Alterations in the alignment of protomers might be sufficient to cause a loss of replicative function. Such subtle alterations have not been examined previously for super-T antigens.

The pattern of binding in region II can be examined by truncating region I with the restriction enzyme *Stu*I. Protected fragments 2 through 4, produced when A protein binds to *Stu*I-cut SV40 DNA, derive entirely from region II (22). When bound to *Stu*I-cut DNA, the SVT2 super-T antigen protects fragments 2 through 4, which are identical to those protected by wild-type A protein. Identical patterns of protected fragments also were observed when 100K super-T antigen and A protein were each bound to a cloned region II sequence (kindly provided by A. DeLucia; V.

Wilson, unpublished observations). This equivalence in pattern and size of protected fragments demonstrates that the 100K super-T antigen binds to region II with the same alignment as that of normal-size A protein.

As a substantial portion of the super-T protein is in association with the host cell p53 protein, it was of interest to examine the binding properties of these complexes. Direct measurements of DNA binding by p53–super-T antigen complexes were obtained by using the p53-specific monoclonal antibodies PAb122 and PAb421. No change in the sizes of the predominant protected fragments was detected when the immunoprecipitation was performed with either anti-p53 monoclonal antibody. This coincidence of size and pattern of protected fragments is consistent with p53–super-T antigen complexes binding in regions I and II with an alignment equivalent to that of free wild-type A protein.

The explanation for the decreased recovery of protected fragments when immunoprecipitation was performed with anti-p53 antibodies is not known. One possibility is that only a fraction of the total protected DNA is protected by p53–super-T antigen complexes, the majority being protected by free super-T antigen. More rigorous determination of the fraction of total super-T antigen associated with p53 will be needed to address this question. A second possibility is that p53–super-T antigen complexes actually differ from free super-T antigen in their DNA-binding ability. The p53 protein might adversely affect the binding of super-T antigen to DNA or the stability of the resulting complexes. Such a quantitative effect is provocative in that it might subtly influence the replication or transcription functions or both of A protein. Less interesting possibilities are that the anti-p53 antibodies have a destabilizing effect on the p53–super-T antigen–DNA complexes or simply are less able to stabilize the super-T antigen–DNA interaction. We and others have noted that the anti-A protein antibodies can enhance the stability of A protein-DNA complexes, perhaps by maintaining the A protein in a particular conformation (17; V. Wilson, unpublished observations). We have found this stabilization to be important for maintenance of the protein-DNA complexes during the multiple wash steps following immunoprecipitation. It is possible that antibodies directed against the p53 protein are simply less able to exert a stabilizing effect on the super-T antigen–DNA complexes, and thus the complexes are more susceptible to dissociation during the washing of the immunoprecipitates.

The in vitro binding results presented here indicate no differences between the overall arrangement of 100K super-T antigen and wild-type A protein on SV40 regions I and II. It is still possible, however, that differences even more subtle could exist, such as alterations in the protein contacts with specific nucleotides (7). Differences of this type have not yet been examined. Further studies will be required to determine whether or not super-T antigens actually constitute a class of A proteins that are replication defective but completely competent for origin binding.

ACKNOWLEDGMENTS

This work was supported by grant MV-206 from the American Cancer Society and by a Biomedical Research Support grant from the National Institutes of Health to Texas A&M University.

LITERATURE CITED

1. **Chandler, D. W., and J. Gralla.** 1980. Specific binding and protection of form II SV40 deoxyribonucleic acid by ribonucleic acid polymerase II from wheat germ. Biochemistry **19:** 1604–1612.
2. **Chaudry, F., G. J. Belsham, and A. E. Smith.** 1983. Biochemical properties of the 145,000-dalton super-T antigen from simian virus 40-transformed BALB/c 3T3 clone 20 cells. J. Virol. **45:**1098–1106.
3. **Chen, S., G. Blanck, and R. Pollack.** 1984. Reacquisition of a functional early region by a mouse transformant containing only defective simian virus 40 DNA. Mol. Cell. Biol. **4:**666–670.
4. **Chen, S., D. S. Grass, G. Blanck, N. Hoganson, J. L. Manley, and R. E. Pollack.** 1983. A functional simian virus 40 origin of replication is required for the generation of a super T antigen with a molecular weight of 100,000 in transformed mouse cells. J. Virol. **48:**492–502.
5. **Clayton, C. E., M. Lovett, and P. W. J. Rigby.** 1982. Functional analysis of a simian virus 40 super T-antigen. J. Virol. **44:**974–982.
6. **Cohen, G. L., P. J. Wright, A. L. DeLucia, B. A. Lewton, M. E. Anderson, and P. Tegtmeyer.** 1984. Critical spatial requirements within the origin of simian virus 40 DNA replication. J. Virol. **51:**91–96.
7. **DeLucia, A. L., B. A. Lewton, R. Tjian, and P. Tegtmeyer.** 1983. Topography of simian virus 40 A protein-DNA complexes: arrangement of pentanucleotide interaction sites at the origin of replication. J. Virol. **46:**143–150.
8. **DiMaio, D., and D. Nathans.** 1980. Cold-sensitive regulatory mutants of simian virus 40. J. Mol. Biol. **140:**129–142.
9. **Gluzman, Y., J. F. Sambrook, and R. J. Frisque.** 1980. Expression of early genes of origin-defective mutants of simian virus 40. Proc. Natl. Acad. Sci. USA **77:**3898–3902.
10. **Gurney, E. G., R. O. Harrison, and J. Fenno.** 1980. Monoclonal antibodies against simian virus 40 T antigens: evidence for distinct subclasses of large T antigen and for similarities among nonviral T antigens. J. Virol. **34:**752–763.
11. **Jones, K. A., R. M. Myers, and R. Tjian.** 1984. Mutational analysis of simian virus 40 large T antigen DNA binding sites. EMBO J. **3:**3247–3255.
12. **Kessler, S. W.** 1975. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. J. Immunol. **115:**1617–1624.
13. **Lovett, M., C. E. Clayton, D. Murphy, P. W. J. Rigby, A. E. Smith, and F. Chaudry.** 1982. Structure and synthesis of a simian virus 40 super-T antigen. J. Virol. **44:**963–973.
14. **May, E., M. Kress, L. Daya-Grossjean, R. Monier, and P. May.** 1981. Mapping of the viral mRNA encoding a super-T antigen of 115,000 daltons expressed in simian virus 40-transformed rat cell lines. J. Virol. **37:**24–35.
15. **May, E., C. Lasne, C. Prives, J. Borde, and P. May.** 1983. Study of the functional activities concomitantly retained by the 115,000 M_r super T antigen, an evolutionary variant of simian virus 40 large T antigen expressed in transformed rat cells. J. Virol. **45:**901–913.
16. **McKay, R.** 1983. Immunoassay for sequence-specific DNA-protein interaction. Methods Enzymol. **92:**138–146.
17. **McKay, R. D. G.** 1981. Binding of a simian virus 40 T antigen-related protein to DNA. J. Mol. Biol. **145:**471–488.
18. **Myers, R. M., and R. Tjian.** 1980. Construction and analysis of simian virus 40 origins defective in tumor antigen binding and DNA replication. Proc. Natl. Acad. Sci. USA **77:**6491–6495.
19. **Reich, N. C., and A. J. Levine.** 1982. Specific interaction of the SV40 T antigen-cellular p53 protein complex with SV40 DNA. Virology **117:**286–290.
20. **Shortle, D. R., R. F. Margolskee, and D. Nathans.** 1979. Mutational analysis of the simian virus 40 replicon: pseudorevertants of mutants with a defective replication origin. Proc. Natl. Acad. Sci. USA **76:**6128–6131.
21. **Tegtmeyer, P., and B. Andersen.** 1981. Partial purification of SV40 A protein and a related cellular protein from permissive cells. Virology **115:**67–74.
22. **Tegtmeyer, P., B. A. Lewton, A. L. DeLucia, V. G. Wilson, and K. Ryder.** 1983. Topography of simian virus 40 A protein-DNA complexes: arrangement of protein bound to the origin of replication. J. Virol. **46:**151–161.
23. **Tegtmeyer, P., M. Schwartz, J. K. Collins, and K. Rundell.** 1975.

Regulation of tumor antigen synthesis by simian virus 40 gene A. J. Virol. **16**:168–178.
24. **Tjian, R.** 1978. The binding site on SV40 DNA for a T antigen-related protein. Cell **13**:165–179.
25. **Tjian, R.** 1979. Protein-DNA interactions at the origin of simian virus 40 DNA replication. Cold Spring Harbor Symp. Quant. Biol. **43**:655–662.
26. **Tooze, J.** 1981. Molecular biology of tumor viruses: DNA tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Wilson, V.G., M. J. Tevethia, B. A. Lewton, and P. Tegtmeyer.** 1982. DNA binding properties of simian virus 40 temperature-sensitive A proteins. J. Virol. **44**:458–466.
28. **Wright, P. J., A. L. DeLucia, and P. Tegtmeyer.** 1984. Sequence-specific binding of simian virus 40 A protein to nonorigin and cellular DNA. Mol. Cell. Biol. **4**:2631–2638.