# Subclasses of simian virus 40 large T antigen: differential binding of two subclasses of T antigen from productively infected cells to viral and cellular DNA

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Two major subclasses of simian virus 40 (SV40) large T antigen were separated by zone velocity sedimentation of crude extracts from productively infected cells. These subclasses, which have been shown to differ biologically and biochemically (Fanning et al., 1981), sedimented at 5–6S and 14–16S. The amount of T antigen in each form was estimated by complement fixation and by immunoprecipitation of T antigen from extracts of cells chronically labeled with [35S]methionine. Each form of T antigen was tested for specific binding to end-labeled restriction fragments of SV40 DNA using an immunoprecipitation assay. The 5–6S and 14–16S forms of T antigen both bound specifically to DNA sequences in the SV40 HindIII C fragment. The sequences required for binding both forms were localized in the same 35-bp region of the origin. However, significant differences in binding activity and affinity for specific and nonspecific DNA were demonstrated. These properties suggest that T antigen subclasses may serve different functions in the lytically infected cell.

Key words: DNA binding/initiation of replication/multifunctional protein/SV40 T antigen

## Introduction

Simian virus 40 (SV40) large tumor antigen (T antigen) is a multifunctional regulatory protein involved in the initiation of each round of viral DNA replication, the regulation of viral transcription, the stimulation of cellular RNA and DNA synthesis and the initiation and maintenance of cellular transformation (reviewed in Tooze, 1980). How the large T antigen performs these varied functions is not yet well understood. Biochemical studies have demonstrated that purified large T antigen and related proteins bind specifically to several sites in the viral genome near the origin of DNA replication (Tjian, 1978; Myers and Tjian, 1980; Shalloway et al., 1980; Tegtmeyer et al., 1981). Genetic evidence indicates that binding of T antigen to at least one of these sites is an essential step in the initiation of viral DNA replication (Shortle et al., 1979; DiMaio and Nathans, 1980). Furthermore, results of in vitro transcription experiments suggest that repression of early transcription involves binding of T antigen to at least two of these sites (Hansen et al., 1981; Myers et al., 1981b).

Nevertheless, an understanding of how T antigen performs its functions requires a better knowledge of the protein itself. Crude T antigen occurs in multiple forms that differ biologically and biochemically (Kuchino and Yamaguchi, 1975; Osborn and Weber, 1975; McCormick and Harlow, 1980; Fanning et al., 1981; Greenspan and Carroll, 1981; Harlow et al., 1981; Bradley et al., 1982). Productively infected cells contain two major forms separable by zone velocity sedimentation: a 5–6S form and a 14–16S form. The 5–6S form serves as a precursor for the faster sedimenting form. The conversion of 5–6S T antigen to the 14–16S form is defective at nonpermissive temperature in tsA-infected cells (Fanning et al., 1981).

It is possible that such subclasses of T antigen may be involved in different functions of the protein. For example, it has been proposed that the process of conversion of 5–6S T antigen to the 14–16S form, or an intermediate in this process, may be correlated with the initiation of SV40 DNA replication (Prives et al., 1980; Fanning et al., 1981). If different subclasses of T antigen do carry out different functions in the lytically infected cell, one might expect their DNA binding properties, which clearly play a central role in the lytic functions of T antigen, to differ.

Thus, subclasses of crude T antigen from lytically infected and transformed cells were assayed for specific binding to SV40 DNA using an immunoprecipitation assay (McKay, 1981). This simple, rapid assay offers several advantages: fresh undenatured cell extracts may be used. T antigen may interact with other components in the extract which stabilize it or modify its properties. Moreover, enrichment for a particular subclass of T antigen and preferential loss of certain subclasses, which may occur during biochemical purification, can be kept to a minimum. The present communication demonstrates that the DNA binding properties of subclasses of crude but authentic T antigen from permissive cells differ in several ways.

## Results

### Specific DNA binding of subclasses of large T antigen

Fresh extract from SV40-infected cells chronically labeled with [35S]methionine was fractionated by zone velocity sedimentation in sucrose density gradients. Each fraction was immunoprecipitated with hamster tumor serum and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography (Figure 1A). Two peaks of large T antigen were observed, one at ~14–16S and another at 5–6S, in agreement with results obtained with shorter labeling times (Fanning et al., 1981). Small t antigen was observed at ~7–8S and appeared to cosediment with a polypeptide slightly smaller than 17 K and two others of 30–50 K. These bands may correspond to the cellular proteins shown to co-immunoprecipitate with small t antigen (Yang et al., 1979).

As a basis for comparing the DNA binding activity in each subclass, the relative amounts of large T antigen in each subclass were estimated by counting the radioactivity in the bands of large T antigen and by a complement fixation assay

(Figure 1B). The 14−16S peak of T antigen, as measured by incorporation of methionine throughout the infection, always contained the bulk of T antigen, in agreement with earlier reports based on Coomassie brilliant blue staining (Weil et al., 1977). However, as measured by the complement fixation assay, the amount of T antigen in the 5−6S subclass appeared to be nearly as great as that in the 14−16S subclass (Figure 1B). A possible explanation for this discrepancy is that each 14−16S oligomer may bind about the same amount of antibody as a 5−6S T antigen molecule, and therefore fix about the same amount of complement. Operationally, the results imply that complement fixation underestimates the relative amount of the 14−16S subclass by a factor of ~3−5.

Sucrose gradient fractions were assayed for specific binding of T antigen to end-labeled restriction fragments of cloned SV40 DNA, using an immunoprecipitation assay (Figure 1C). Fractions 12−17 and 20−22 bound specifically to HindIII C fragment, which includes the SV40 origin of replication and sites bound specifically by purified T antigens.

The 5−6S T antigen bound more HindIII C fragment than did T antigen in the 14−16S form. The fast-sedimenting shoulder on the 14−16S peak of T antigen, which contains T antigen complexed with host p53 phosphoprotein (Fanning et al., 1981; Greenspan and Carroll, 1981; Harlow et al., 1981), also bound specifically to the viral HindIII C fragment. Similar results have been observed with T antigen-p53 complexes from SV40-transformed cells (Reich and Levine, 1982; C. Burger and E. Fanning, in preparation).

### Characterization of the DNA binding assay

The sucrose gradient fractions containing 5−6S and 14−16S T antigen were each pooled and tested for specific binding to cloned SV40 DNA (pSVWT) and to cloned SV40 HindIII C fragment (pEP392) (Figure 2A). Both forms of T antigen bound specifically to unit length viral DNA and to HindIII C fragment. The HindIII C fragment was retained in the immune complex somewhat better than unit length SV40 DNA, possibly due to its smaller size. Crude extract from infected cells but not from uninfected TC-7 cells showed specific binding to viral DNA in this assay. No DNA was pre-
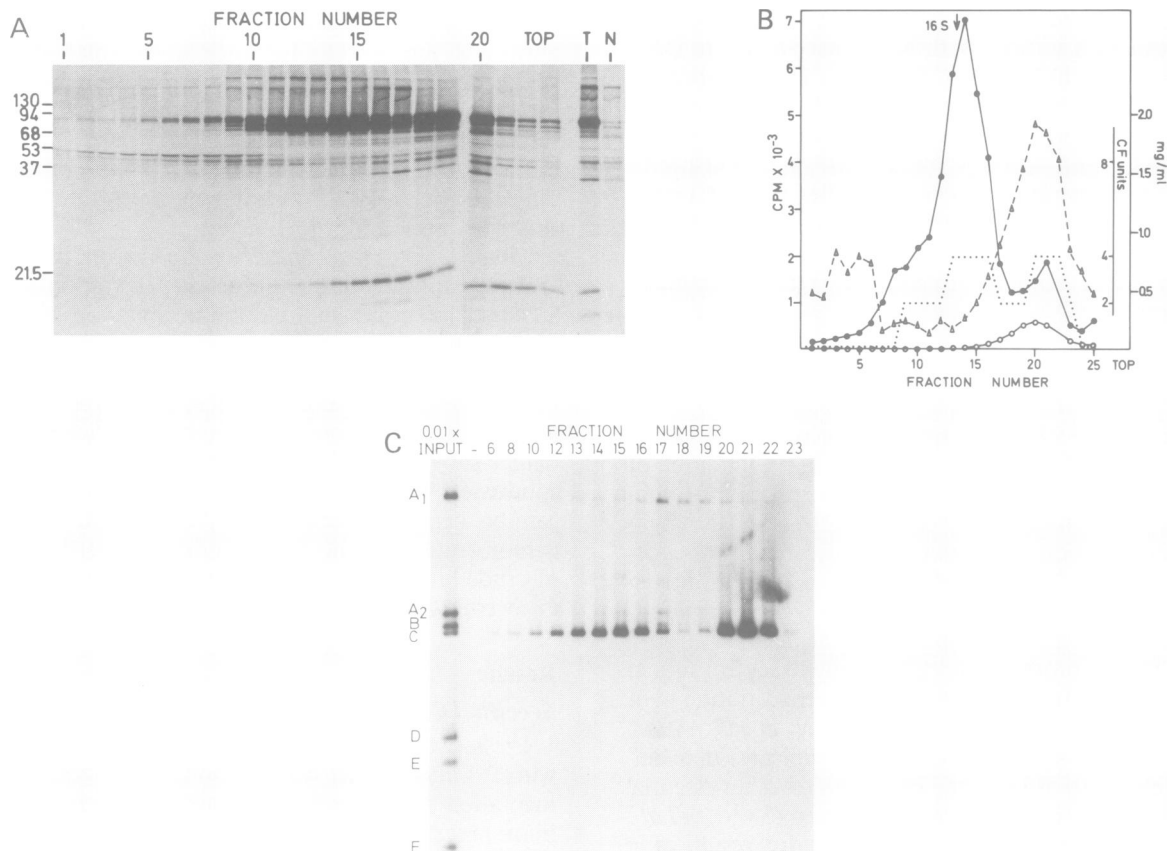


Fig. 1. Separation of subclasses of SV40 T antigen by zone velocity sedimentation. SV40-infected TC-7 cells were labeled with 50 μCi/ml of [$^{35}$S]methionine 2−40 h after infection (A) and in a separate experiment, 2−42 h after infection (B) in 80% methionine-free medium, 20% DME, 2% calf serum. Crude cell extract (200 μl) was layered on sucrose density gradients and centrifuged. (A) Each fraction of the gradient was immunoprecipitated with hamster tumor serum. The immunoprecipitated proteins were separated by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970) and visualized by fluorography. Aliquots (10 μl) of crude cell extract were immunoprecipitated with tumor serum (T) and normal hamster serum (N) and analyzed in parallel. The positions of marker proteins of known mol. wts. are shown on the left. The peak of SV40 form II marker DNA (16S) was in fractions 13−14 of a parallel gradient. (B) A 25 μl aliquot of each fraction was monitored for T antigen by CF as described in Materials and methods. Protein concentration was determined in a 10 μl aliquot of each fraction as described (Schaffner and Weissmann, 1973). The remainder of each fraction was immunoprecipitated with hamster tumor serum and analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. The bands of large T antigen (84−90 K) and small t antigen (17 K) were excised from the gels, solubilized and counted. The peak of SV40 form II DNA run in a parallel gradient was in fractions 13−14. ●——●, large T; ○——○, small t; •••, CF units per 25 μl; △——△, protein concentration, mg/ml. (C) T antigen from a gradient similar to that in (A) and (B) (50 μl of each fraction) was tested for binding to $^{32}$P-end-labeled HindIII fragments of pSVWT DNA (0.2 μg), as described in Materials and methods. A small aliquot (1%) of the labeled DNA used in the binding assays was electrophoresed in parallel.
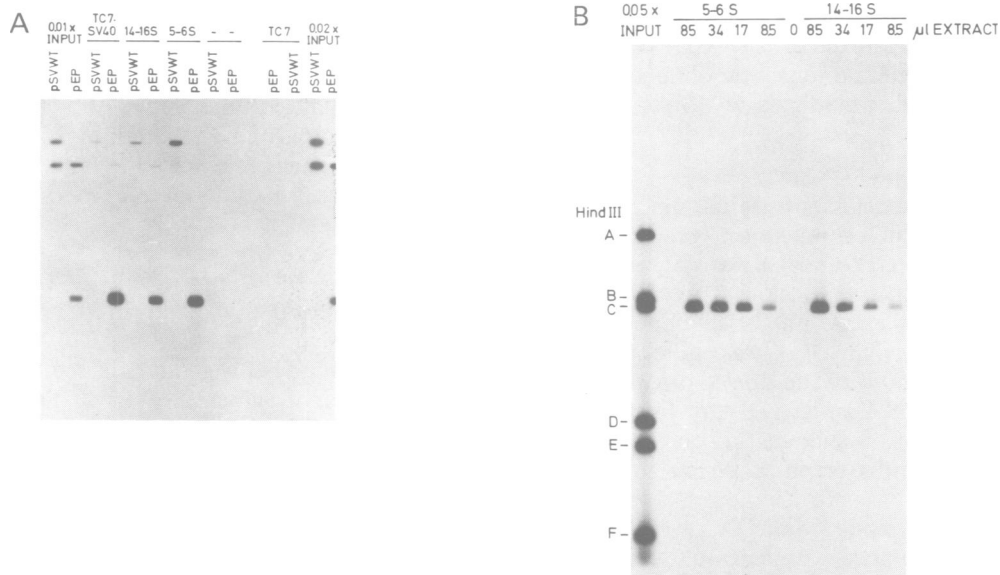
**Fig. 2.** Specific DNA binding of subclasses of T antigen in an immunoprecipitation assay. (A) Extract from SV40-infected TC-7 cells was fractionated by zone velocity sedimentation. The 5−6S and 14−16S fractions were each pooled and 75 µl of each pool was assayed for binding to end-labeled *Bam*HI fragments of pSVWT DNA (0.35 µg per assay). Control assays were performed with 100 000 g supernatant of crude extract from infected and uninfected cells, and without extract. Small aliquots (1% and 2%) of the DNA used in the binding assays were run on the same gel. (B) Different amounts of the 14−16S and 5−6S extracts were assayed for binding of T antigen to SV40 *Hind*III DNA fragments (0.2 µg per assay), as described in Materials and methods.

cipitated specifically in control assays performed with tumor serum but without T antigen extract. Control assays carried out with normal hamster serum instead of tumor serum precipitated no SV40 DNA (not shown).

Different amounts of 14−16S and 5−6S T antigen extract were tested for specific binding to SV40 DNA. Assuming that DNA, anti-SV40 tumor serum, and *Staphylococcus aureus* are all present in excess over the amount of T antigen in the assay, the amount of DNA bound should be directly proportional to the amount of T antigen extract added. The amount of *Hind*III C fragment precipitated in the binding assay was, in fact, dependent on the amount of T antigen extract added (Figure 2B). When the bands of *Hind*III C fragment were excised from the gel and counted, the amount of radioactivity increased approximately linearly with the volume of T antigen extract added to the assay (not shown).

### T antigen from infected and transformed cells: quantitative differences in SV40 DNA binding

SV40-transformed SV80 cells contain at least three major forms of T antigen separable by sedimentation: 5−6S and 14−16S forms similar to the corresponding forms from productively infected cells and a 23−25S form composed of highly phosphorylated T antigen complexed with host phosphoprotein p53 (Fanning *et al.*, 1981). These three forms and the 5−6S and 14−16S forms from productively infected cells were assayed for specific binding to *Hind*III fragments of SV40 DNA (Figure 3). An equal number of complement fixation (CF) units of T antigen was added to each assay.

The 5−6S form of T antigen from productively infected cells bound two to four times more *Hind*III C fragment than did 14−16S T antigen. Since the actual amount of T antigen in the 14−16S form is probably underestimated by the complement fixation assay (Figure 1B), it appears that 5−6S T antigen binds to the *Hind*III C fragment up to 10-fold more efficiently than the 14−16S form. These observations could
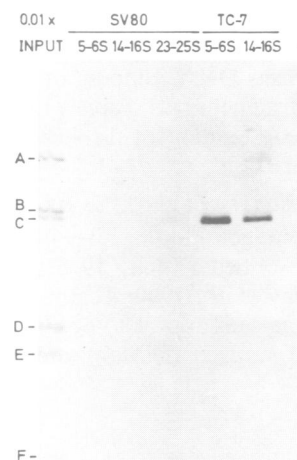


**Fig. 3.** DNA binding activity of T antigen extracts from SV80 cells and SV40-infected TC-7 cells. Crude cell extracts from 10⁷ SV80 cells and from 10⁷ infected TC-7 cells were fractionated by zone velocity sedimentation as described in Materials and methods. The 5−6S, 14−16S, and 23−25S (SV80) fractions were pooled. From each pool, 160 µl containing approximately equal CF units of T antigen were incubated with 0.2 µg of labeled *Hind*III fragments of SV40 DNA. The bound DNA was immunoprecipitated with hamster tumor serum and analyzed as described in Materials and methods. A control assay without cell extract was analyzed in parallel. Microdensitometry scans of the autoradiograms indicated that 5−6S lytic T antigen bound twice as much *Hind*III C fragment as 14−16S T antigen, which in turn bound >13 times as much as the SV80 T antigen subclasses.

also be explained by a phosphatase activity in the 14−16S fraction which removes the 5' end label from the DNA. This possibility is unlikely, however, because under the conditions of the assay, trace amounts of phosphatase activity could be found only in the 5−6S extract (C. Burger and E. Fanning, unpublished data). T antigen from SV80 cells bound at least

10-fold less *Hind*III C fragment than an equivalent amount of T antigen from productively infected cells. After these experiments were completed, similar results were obtained with purified SV80 T antigen in a filter binding assay (Myers *et al.*, 1981c).

### Binding of T antigen to viral and cellular DNA

The affinity of T antigen subclasses for viral and cellular DNA was examined in rate competition experiments. Samples of 5–6S and 14–16S T antigen extract were pre-incubated with labeled fragments of cloned SV40 DNA. Three hours after addition of excess unlabeled cloned viral DNA, ~20% of the labeled origin DNA was still bound to the 5–6S T antigen, while ~50% of the labeled origin DNA remained bound to the 14–16S T antigen (Figure 4A). These results indicate that the 14–16S subclass has an equal or somewhat greater affinity for the origin sequences than the 5–6S form.

When labeled fragments of cloned viral DNA were added to T antigen extracts pre-incubated with a 50-fold excess of unlabeled cellular DNA, most of the 5–6S T antigen dissociated from cellular DNA within 2 h, and bound specifically to labeled origin DNA, whereas most of the 14–16S T antigen remained bound to the unlabeled cellular DNA (Figure 4B). These results imply that the affinity of the 14–16S T antigen for cellular DNA must be significantly greater than that of the 5–6S T antigen.

This interpretation was confirmed in equilibrium competition experiments. T antigen extract was incubated with labeled cloned viral DNA fragments mixed with different amounts of unlabeled calf thymus DNA. Binding of 5–6S T antigen to the labeled origin fragment was scarcely affected by excess cellular DNA, whereas binding of 14–16S T antigen was clearly reduced (Figure 4C).

### SV40 DNA binding site(s) of subclasses of lytic T antigen

Several distinct T antigen binding sites in the SV40 origin region have been described (Tjian, 1978,1979; Myers and Tjian, 1980; Shalloway *et al.*, 1980; Tegtmeyer *et al.*, 1981). These different binding sites appear to be correlated with different functions of T antigen (DiMaio and Nathans, 1980)

and might therefore be recognized by different subclasses of T antigen. Cloned SV40 DNAs carrying deletions in previously described T antigen binding sites were thus cleaved with *Hind*III, end-labeled, and assayed for binding to the 5–6S and 14–16S forms of T antigen from infected cells. Deletions of late DNA sequences reported to bind to D2-T antigen and to SV80 T antigen did not reduce the amount of SV40 *Hind*III C fragment bound by either subclass of T antigen (Figure 5A). Moreover, neither the 325-bp *Hae*III fragment (nucleotides 6– 331) nor the 286-bp *Alu*I fragment (5226–271) bound to T antigen (C. Burger, unpublished data). The sequences between the *Bgl*I site and the *Hind*III site at nucleotide 5171 were investigated by using end-labeled *Dde*I restriction fragments of SV40 DNA in the binding assay. A 310-bp fragment, which contains D2 binding site I and sequences between sites I and II, was recognized by both forms of T antigen (Figure 5B). Furthermore, binding of both forms of T antigen to the same *Alu*I fragment of pdl1209 DNA (Figure 5B) suggests that the 35 bp of DNA between the *Alu*I site at nucleotide 5226 and the dl1209 deletion end point at or near the *Hae*III site at nucleotide 5191 contain the major binding site(s) for both forms of T antigen from permissive cells (Figure 5C).

### Discussion

Subclasses of SV40 large T antigen from productively infected cells have been shown to bind specifically to the origin region of SV40 DNA. The 5–6S form of T antigen appears to bind more 'efficiently' to the viral origin region than the 14–16S form (Oren *et al.*, 1980; Gidoni *et al.*, 1982; Figure 1). Several different properties of each subclass which could contribute to this observation have been investigated: the fraction of T antigen molecules able to bind specifically to origin DNA (i.e., binding activity), the affinity of T antigen for origin DNA, its affinity for nonspecific DNA, and the DNA sequences required for origin binding. The origin DNA binding activity of 5–6S T antigen was ~10-fold greater than that of the 14–16S subclass (Figure 3). However, the affinity of 14–16S T antigen for origin DNA was equal to or
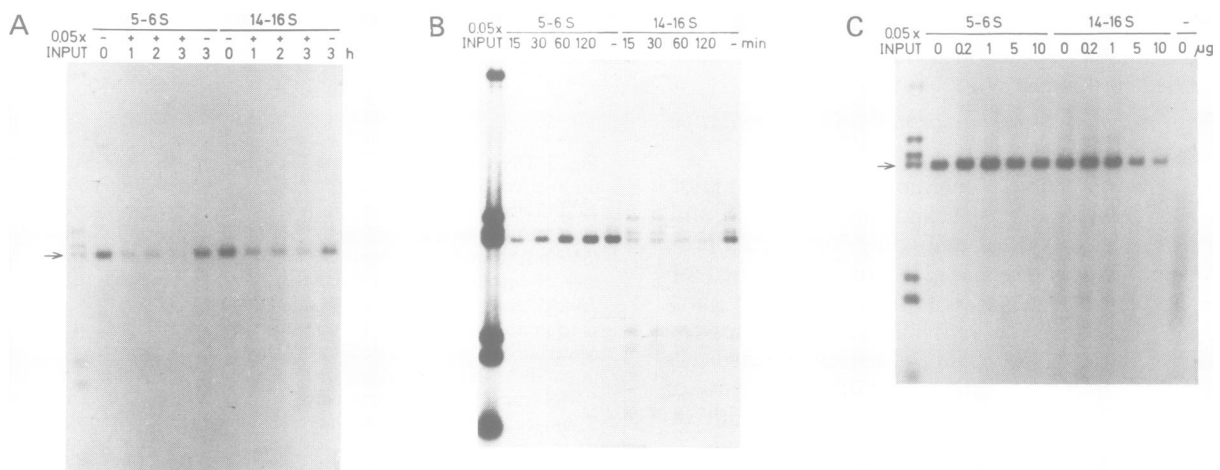


**Fig. 4.** Affinity of T antigen subclasses for viral and cellular DNA. Crude extract from SV40-infected TC-7 cells was fractionated by zone velocity sedimentation. (**A**) Samples (30 μl) of the 5–6S and 14–16S fractions were incubated for 15 min with 0.2 μg end-labeled *Hind*III fragments of SVWT DNA. A 30-fold excess of unlabelled pSVWT DNA was added to the samples indicated (+) and bound DNA was immunoprecipitated after 1, 2, and 3 h. Arrow denotes the SV40 *Hind*III C fragment. (**B**) Samples of T antigen extract were incubated with 10 μg of unlabeled calf thymus DNA except where indicated (–). After 15 min, 0.2 μg end-labeled *Hind*III restricted pSVWT DNA was added. Bound DNA was immunoprecipitated after 15, 30, 60, and 120 min. (**C**) Samples of T antigen extract were incubated with 0.2 μg end-labeled *Hind*III fragments of pSVWT DNA in the presence of different amounts of unlabeled calf thymus DNA, as indicated. Bound DNA was immunoprecipitated after 15 min. Arrow shows the *Hind*III C fragment.
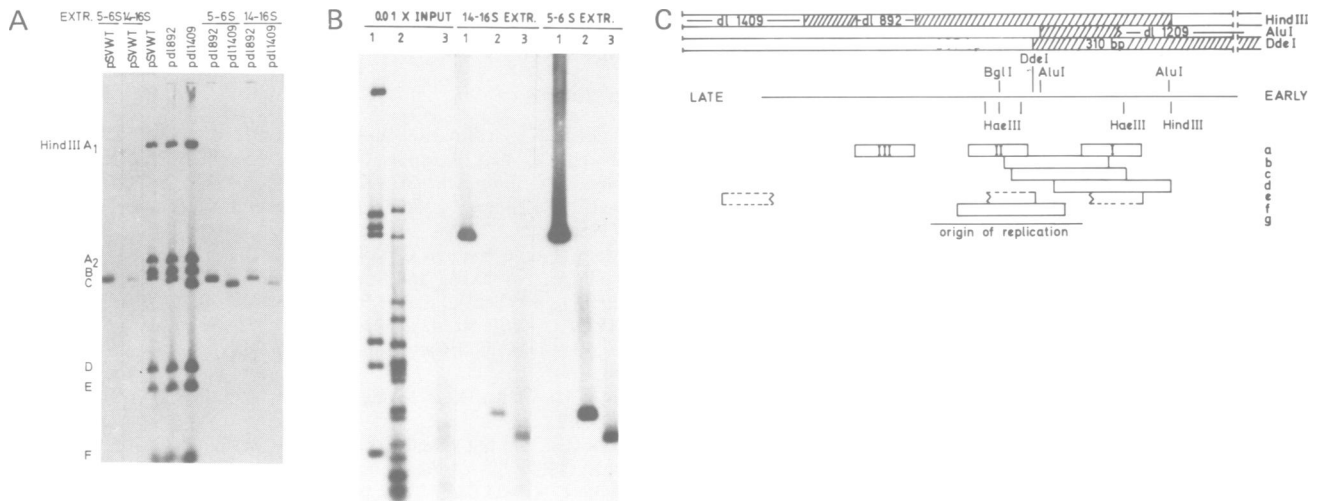
Fig. 5. SV40 DNA sequences required for binding of subclasses of T antigen. (A) Subclasses of T antigen prepared by zone velocity sedimentation were assayed for specific binding to end-labeled HindIII fragments of pd1892, pd11409, and pSVWT DNA (0.25 μg of DNA and 80 μl of T antigen extract per assay) as described in Materials and methods. Aliquots (5%) of the DNAs used in the binding assays were electrophoresed in parallel. (B) Subclasses of T antigen were assayed for specific binding to end-labeled DdeI fragments of SV40 DNA (2), AluI fragments of pd11209 DNA (3) and for comparison, HindIII fragments of pSVWT DNA (1) (0.2 μg of DNA and 90 μl of T antigen extract per assay). Aliquots of the DNA used in the binding assays were electrophoresed in parallel. Part of the sample in lane 3, left, was lost during loading. (C) Diagram of SV40 DNA fragments required for T antigen binding in this assay. The shaded areas represent those DNA sequences bound by T antigen in (A) and (B) above. SV40 DNA binding sites defined previously are shown for comparison (a) Tjian, 1978,1979; (b) Myers and Tjian, 1980; (c) McKay, 1981; (d) McKay and DiMaio, 1981; (e) Shalloway et al., 1980; (f) Tegtmeyer et al., 1981; (g) DiMaio and Nathans, 1980).

slightly greater than that of the 5−6S form (Figure 4A), but its affinity for cellular DNA was much greater (Figure 4B,C). Both forms of T antigen required the same DNA sequence for specific binding.

Earlier observations also support the conclusion that 14−16S T antigen binds to nonspecific DNA more tightly than 5−6S antigen. Highly phosphorylated SV80 T antigen, which exists primarily in oligomeric form (Fanning et al., 1981), binds more tightly to calf thymus DNA cellulose than the less phosphorylated form (Montenarh and Henning, 1980), although SV80 T antigen is defective in origin specific binding (Figure 3). Furthermore, 14−16S lytic T antigen elutes from phosphocellulose columns at higher salt concentration than 5−6S T antigen (A. Dorn, D. Brauer, E. Fanning, and R. Knippers, in preparation).

The DNA binding properties reported here have been confirmed using partially purified subclasses of T antigen in a filter binding assay (Dorn et al., in preparation). The two forms differ in their optimal salt and pH conditions for specific binding to SV40 DNA. Under optimal conditions, the affinity of 5−6S T antigen for origin DNA relative to that for bacterial plasmid DNA is much greater than the relative affinity of the 14−16S T antigen for origin DNA. On the other hand, binding of the 14−16S T antigen is more resistant to high pH and salt concentration.

Subclasses of T antigen whose DNA binding properties differ have been described previously. Newly synthesized 5−7S T antigen from permissive cells binds more tightly to SV40 multi-origin DNA cellullose than 'aged' oligomeric T antigen (Oren et al., 1980; Gidoni et al., 1982). Thus, one might expect the affinity of 5−6S T antigen for origin DNA to be significantly greater than that of 14−16S T antigen. On the contrary, the affinity of the 5−6S T antigen for origin DNA in solution is somewhat lower than that of the 14−16S form (Figure 4A). Thus, we believe that the more 'efficient' origin binding of 5−6S T antigen may derive solely from its higher binding activity (more active molecules) and its much lower

affinity for nonspecific DNA sequences, rather than from higher affinity for origin DNA.

D2-T antigen has been observed in three forms: a very large aggregate which did not bind to DNA, a smaller aggregated form that bound specifically to origin DNA and a still smaller form that bound to DNA in a nonspecific manner (McKay, 1981; Myers et al., 1981a). This conclusion appears to be at variance with both the present results and those of Gidoni et al. (1982), assuming that the 5−6S T antigen corresponds to the smallest form of D2 and the 14−16S T antigen to the intermediate form of D2. The different conditions used for binding and immunoprecipitation in the studies of D2-T and in this report might be responsible for the different results. It is also possible that D2-T antigen aggregation and DNA binding properties differ subtly from those of authentic T antigen. Indeed, D2-T antigen appears to be defective in initiation of viral DNA replication (A. Graessmann, personal communication).

The DNA binding properties of T antigen demonstrated here and elsewhere (Dorn et al., in preparation) suggest that the two forms of T antigen may in fact function differently. The data would be consistent with the idea that specific binding of T antigen to the origin may take place in several steps. As a working model, we suggest that newly synthesized 5−6S T antigen, possibly a dimer (Bradley et al., 1982), first binds transiently to DNA, scanning the intracellular DNA until the origin sequence is recognized and bound specifically, as proposed for the lac repressor-DNA interaction (Winter et al., 1981). T antigen may then aggregate, possibly on the DNA, into a 14−16S form which is more firmly bound to the DNA. The aggregation process appears to be essential for the initiation of replication (Fanning et al., 1981). Phosphorylation, perhaps coupled with aggregation of T antigen (E. Baumann and R. Hand, in preparation), may regulate the amount of newly synthesized T antigen available for specific binding to the origin. Replication could be initiated on those DNA molecules which carry T antigen on the major binding site

and which then become transcriptionally activated (reviewed in Hobom, 1981). Although many features of this proposal have not yet been tested, it summarizes the data available and may serve as a useful basis for further experimentation.

## Materials and methods

The materials and procedures used in this study, except for those below, have been described previously (Fanning et al., 1981).

### Complement fixation assay (Sever, 1962)

A 25 μl sample of each fraction of the sucrose gradient was assayed for T antigen. Two-fold dilutions of pooled hamster serum and guinea pig complement (Behringwerk) were added to each well and incubated for 18–20 h at 4°C. A suspension of sensitized sheep red blood cells (Behringwerk) was then added to each well and the highest dilution of extract at which the erythrocytes were not lysed was taken as the number of CF units of T antigen per 25 μl of extract.

### DNA

SV40 form I DNA was prepared from SV40-infected TC-7 cells at 60 h after infection by Hirt extraction (Hirt, 1967) and equilibrium density centrifugation in CsCl-ethidium bromide gradients. The following plasmid DNAs were used under conditions (L2/B1) approved by the Biological Safety Committee of the Bundesgesundheitsamt: pEP392, the SV40 HindIII C fragment cloned in the HindIII site of the vector pAT153 (Twigg and Sherratt, 1980) and made available to us by P.W.J. Rigby and E. Paucha, Imperial College, London; pSVWT, wild-type SV40 DNA cloned in the BamHI site of pAT153; pd1892 and pd11409, viable deletion mutants (Shenk et al., 1976; Shenk, 1977) cloned in the BamHI site of pAT153. Deletion pd11209, a defective SV40 deletion mutant derived by partial digestion with HaeIII (Cole et al., 1977) and cloned in the BamHI site of pBR322, was supplied by C.N. Cole, Yale University, New Haven. Plasmid DNA was prepared from cleared lysates of Escherichia coli (Hb101) essentially as described (Wensink et al., 1974). SV40 DNA and recombinant plasmid DNAs were cleaved with restriction endonuclease (Boehringer, Mannheim and Bethesda Research Laboratories) according to the supplier's instructions. The fragments were treated with alkaline phosphatase and 5' end-labeled with T4 polynucleotide kinase (Boehringer, Mannheim) and [γ-³²P]ATP (Amersham) as described (Chaconas et al., 1975; Maxam and Gilbert, 1980).

### DNA binding assay

The peak fractions of T antigen (0.4–0.5 ml) from sucrose gradients were pooled. Samples to be assayed, typically 20–80 μl, were adjusted to 10 mM Hepes (pH 7.8), 80 mM KCl, 0.5 mM MgCl₂, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride (assay volume 200–500 μl). These conditions, which are suboptimal for T antigen binding to DNA, were chosen to minimize the nonspecific binding which occurs at lower pH and salt concentration (Dorn et al., in preparation). End-labeled fragments of SV40 DNA or recombinant DNA (0.2–0.5 μg) were added to each sample of T antigen extract and incubated at 0°C for 15 min. Heat-inactivated (56°C, 30 min) anti-SV40 hamster tumor serum (5–20 μl) was added and incubated at 0°C for 1 h. Immunoprecipitation of bound DNA was also effective with rabbit antiserum against native D2-T antigen and somewhat less effective with mouse anti-SV40 tumor serum, but rabbit antiserum against denatured large T antigen was unsatisfactory in this assay. A 4-fold volume of fixed S. aureus, which had been washed at least five times in 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 150 mM NaCl, 0.05% Nonidet P-40 (NET) and resuspended in NET (10% v/v) was added and incubated for 15 min at 0°C. The immune complexes were pelleted, and washed two to three times with 0.5 ml of NET. Washing the immune complexes with NET rather than binding buffer did not affect the binding of T antigen to wild-type SV40 origin region DNA. The final pellet was resuspended in 20 μl double-strength sample buffer (Laemmli, 1970), incubated for 15 min at 65°C and centrifuged. The supernatant was loaded on horizontal 2% agarose gels in 40 mM Tris-acetate (pH 7.8), 5 mM Na-acetate, 1 mM EDTA and electrophoresed. The gel was then treated with industrial ethanol for 30 min, dried and exposed to Kodak BB5 film at −70°C.

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