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Topography of Simian Virus 40 A Protein-DNA Complexes: Arrangement of Pentanucleotide Interaction Sites at the Origin of Replication

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Investigation of the DNA binding properties of the simian virus 40 (SV40) A protein (large T antigen) and the hybrid adenovirus-SV40 D2 protein revealed that both viral proteins protect similar regions of SV40 DNA from digestion by DNase I or methylation by dimethyl sulfate. However, the interaction of D2 protein with DNA was more sensitive to increases of NaCl concentration than was the interaction of wild-type SV40 A protein. Dimethylsulfate footprinting identified 13 DNA pentanucleotide contact sites at the viral origin of replication. The sequences of these sites corresponded to the consensus family 5'-(G>T)(A>G)GGC-3'. The pentanucleotides were distributed in three regions of origin DNA. Region I contained three pentanucleotide contact sites arranged as direct repetitions encompassing a span of 23 base pairs. In region II, four pentanucleotides were oriented as inverted repetitions that also spanned a total of 23 base pairs. Region III had six recognition pentanucleotides arranged as direct repetitions in a space of 59 base pairs. These fundamental variations in DNA arrangement are likely to determine different patterns of protein binding in each region.

The simian virus 40 (SV40) gene *A* product is a multifunctional protein that regulates the viral growth cycle (7, 28, 29). Genetic studies have indicated that SV40 A protein (large T antigen) directs the initiation of viral DNA replication (3, 20) from a 65-base pair (bp) origin centered at the unique *Bgl*I restriction site of SV40 DNA (4). Additional adjacent sequences may also be involved as ancillary regions for the enhancement of replication (1). Several studies indicate that SV40 A protein must bind to the origin to initiate DNA replication (10, 12, 18, 30). In addition, SV40 early transcription is under autogenous regulation by A protein (13, 24). Current in vitro studies suggest that the A protein acts as a repressor of viral early gene transcription by binding to the early promoter that overlaps the origin of replication (8, 11, 15).

A number of studies have described the binding of SV40 A protein to the origin region (11, 16, 22, 25–27). Tjian and co-workers have used A protein coded by D2 or R26 hybrid adenovirus-SV40 and purified from HeLa cells (11, 26, 27). They described tandem binding to regions I, II, and III at the origin of replication. The original mapping suggested that the three regions were similar in size. Shalloway et al. (16) used a partially defective A protein purified from the SV80 line of transformed human cells and described binding to DNA extending far to the late side of Tjian's region III. Tegtmeyer et al. (22) purified wild-type A protein from acutely infected permissive cells. They found that regions I and II apparently contained multiple binding sites and that binding to regions I and II resulted in different extents of DNase protection. In the present study, we compare the DNA binding properties of the wild-type A protein and the more commonly used hybrid D2 protein under standardized conditions.

Dimethyl sulfate (DMS) protection studies by Tjian (27) identified a pentanucleotide important for A protein binding. However, that sequence did not account for binding in all three regions. Careful analysis of DMS protection under a variety of conditions now allows identification of a family of pentanucleotides that directs binding in all regions. The arrangement of the pentanucleotides is especially provocative because it is substantially different in each of the three binding regions. These fundamental variations in DNA arrangement are likely to determine differ-

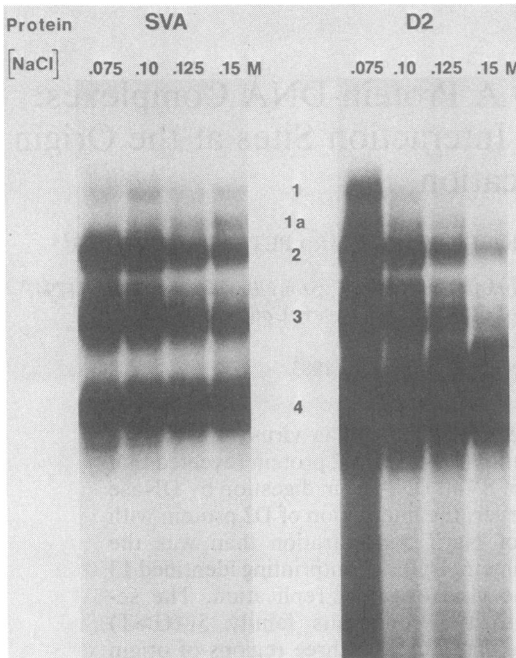

FIG. 1. Fragments of SV40 DNA protected from nuclease digestion by wild-type SV40 A (SVA) or D2 protein at various NaCl concentrations. Nick-translated DNA (2 ng) was mixed with SV40 A protein (0.01 μg) or D2 protein (0.1 μg) in 100 μl of PIPES binding buffer containing the NaCl molar concentrations listed above the gel lanes. DNA binding, DNase treatment, and analysis of DNA fragments are described in the text. The four major size classes of protected SV40 DNA are labeled as 1, 2, 3, and 4 in order of decreasing molecular weights. Fragment 1a is an additional fragment protected only at high salt concentrations.

ent patterns of SV40 A protein binding within each region and may determine functional differences as well.

MATERIALS AND METHODS

Protein purification. SV40 A protein was purified from SV40-infected CV-1 cells (American Type Culture Collection) as previously described (21). This method provides partially purified wild-type SV40 A protein assessed to be approximately 40% pure. The adenovirus-SV40 (Ad2$^+$D2) hybrid D2 protein, purified >95% (26), was a generous gift from R. Myers.

DNase fragment assay. SV40 DNA, isolated and purified according to Spillman et al. (19), was nick translated (14) by using an Amersham Corp. commercial kit. In the binding reaction SV40 A protein (0.01 μg) or D2 protein (0.1 μg) was mixed with 2 ng of SV40 DNA in 100 μl of PIPES [piperazine-*N*,*N*′-bis(2-ethane-sulfonic acid)] binding buffer (0.02 M PIPES, 0.0001 M EDTA, 0.001 M dithioerythritol, 10% glycerol, 10 μg of bovine serum albumin per ml) and various concentrations of NaCl at pH 7.0. The DNA-protein mixture was held for 1 h at 4°C, and then 5 U of pancreatic DNase I (Worthington Diagnostics) in 5 μl of PIPES binding buffer with 0.5 M $MgCl_2$ and 0.05 M $CaCl_2$ was added for 5 min at 4°C. The enzymatic digestion as stopped by the addition of 1 ml of PIPES binding buffer containing 0.01 M EDTA. The DNA bound to protein was rapidly isolated on nitrocellulose filters (13 mm, 0.45 μm; Schleicher & Schuell) and washed five times with PIPES binding buffer containing 0.1 M NaCl and five times with PIPES binding buffer containing 0.001 M NaCl. Filters were then transferred to 1.5-ml microfuge tubes containing 50 μl of 0.01 M Tris-borate (pH 8.3)–0.2% sodium dodecylsulfate–10% glycerol–0.001 M EDTA. After elution for 1 h at room temperature, protected fragments were analyzed by electrophoresis, using 12% nondenaturing polyacrylamide gels (2) in a 32-cm Hoefer constant-temperature apparatus at 20°C for 4 h at 750 V.

DNase footprinting. The *Hin*fI-*Bst*NI origin-containing fragment of SV40 DNA was used for DNase footprinting. To radiolabel the 3′ early end, SV40 DNA was digested with *Hin*fI and simultaneously labeled with [^{32}P]dTTP (specific activity, >3,000 Ci/mmol; New England Nuclear) by use of avian myeloblastosis virus reverse transcriptase in 0.05 M Tris (pH 8.0)–0.05 M KCl–0.005 M $MgCl_2$–0.001 M dithioerythritol–100 μg of bovine serum albumin per ml. The transcriptase was inactivated by heating for 10 min at 70°C, and the labeled DNA was then digested by adding *Bst*NI. The origin-containing fragment was purified by gel electrophoresis as described by Tegtmeyer et al. (22). To label the 3′ late end, the order of the restriction enzymes used above was reversed. For the footprinting, SV40 A protein (0.025 μg) or D2 protein (0.25 μg) was bound to 0.5 ng of the 270-bp DNA fragment in 100 μl of PIPES binding buffer with various concentrations of NaCl and also with 10 ng of unlabeled pBR322 DNA. After binding for 1 h at 4°C, 5 $\times 10^{-4}$ U of DNase I in 5 μl of PIPES binding buffer with 0.1 M $MgCl_2$ and 0.05 M $CaCl_2$ was added for 5 min at 4°C. The DNase activity was stopped by the addition of 105 μl of 1 M NH_4-acetate–0.2% sodium dodecyl sulfate–0.1 M EDTA, pH 6.0. The DNA was then precipitated with 95% ethanol and analyzed by electrophoresis, using 8.3 M urea–8% polyacrylamide gels (9) at a constant temperature of 55°C.

DMS footprinting. SV40 A (0.05 μg) or D2 (0.5 μg) protein was bound to the end-labeled *Hin*fI-*Bst*NI fragment (0.5 ng with 10 ng of unlabeled pBR322 DNA) as described for the DNase footprinting. After binding, 1 μl of 10 M DMS (Aldrich Chemical Co.) was added to the sample for 10 min at 4°C. The DMS reaction was stopped, and the DNA was rapidly processed according to the Maxam and Gilbert DNA sequencing method specific for cleavage at guanine residues (9). The cleaved DNA was analyzed by 8.3 M urea–8% polyacrylamide gel electrophoresis and autoradiography.

Restriction enzymes. All enzymes were obtained from New England Biolabs.

RESULTS

DNase fragment assay. Previous studies have shown that the SV40 A protein protects several classes of origin DNA from complete digestion by DNase (22, 26). However, the reports were not in complete agreement on the number, size,

and genetic location of the protected DNA fragments. The variations seemed likely to reflect the use of either different protein preparations or different assay conditions. To resolve these apparent discrepancies and to extend our understanding of the protein-nucleic acid binding pattern, we have compared proteins independently purified in two laboratories under a variety of in vitro binding conditions.

Purified SV40 DNA was nick translated and used in an in vitro binding reaction with SV40 A protein or D2 protein. The protein-DNA complexes were isolated and subjected to nuclease digestion so that any nucleotides not protected by bound protein would be completely hydrolyzed from the DNA molecule. Protected DNA fragments were displayed by polyacrylamide gel electrophoresis and autoradiography.

Figure 1 presents a comparison of the DNA fragments protected by the SV40 A protein or the D2 protein. As reported earlier (22), the wild-type SV40 A protein preparation protects four major size classes of SV40 DNA under all conditions shown. The classes have fragment lengths of 105 to 110, 60 to 65, 40 to 45, and 30 to 35 bp. At NaCl concentrations >0.1 M, a 70- to 75-bp 1a fragment is also protected. Each class is somewhat heterogeneous in molecular weight, and classes 3 and 4 are known to be derived from two regions of DNA (23). The DNA binding pattern of the D2 protein closely resembles that of the SV40 A protein. However, DNA binding of the D2 protein is more sensitive to high salt concentrations. At 0.15 M NaCl, protection of classes 1 and 3 decreases, whereas class 4 becomes more heterogeneous. The result is an altered protection pattern that resembles the findings first reported by Tjian, who used D2 protein under similar conditions (26). However, Tjian described the 70- to 75-bp class 1a DNA but not the 60- to 65-bp class 2 DNA that is also present in this and other studies (22, 23). We do not know the explanation for this difference but presume that subtle differences in conditions may favor the protection of class 1a rather than class 2 DNA. In any event, mapping of all of the protected fragments provides important information on the arrangement of A protein bound to the origin (23).

Since data in Fig. 1 show protected DNA fragments of equivalent molecular weight for both SV40 A protein and D2 protein, a simple mapping technique was used to verify that the location of these fragments from the SV40 genome is equivalent. *BglI* endonuclease cuts SV40 DNA at a single site within the A protein binding region. When SV40 DNA is digested with *BglI* endonuclease and then presented to SV40 A protein or D2 protein in a DNase protection assay, it is apparent that fragments 1 and 2 are no longer protected (Fig. 2). Fragment 3 also becomes less heterogenous. New DNA fragments (see arrows, Fig. 2) that appear after protection by either SV40 A protein or D2 protein are identical. These fragments have been mapped in the accompanying manuscript (23). We conclude that SV40 A and D2 proteins bind origin DNA in a similar arrangement at NaCl concentrations lower than 0.125 M.

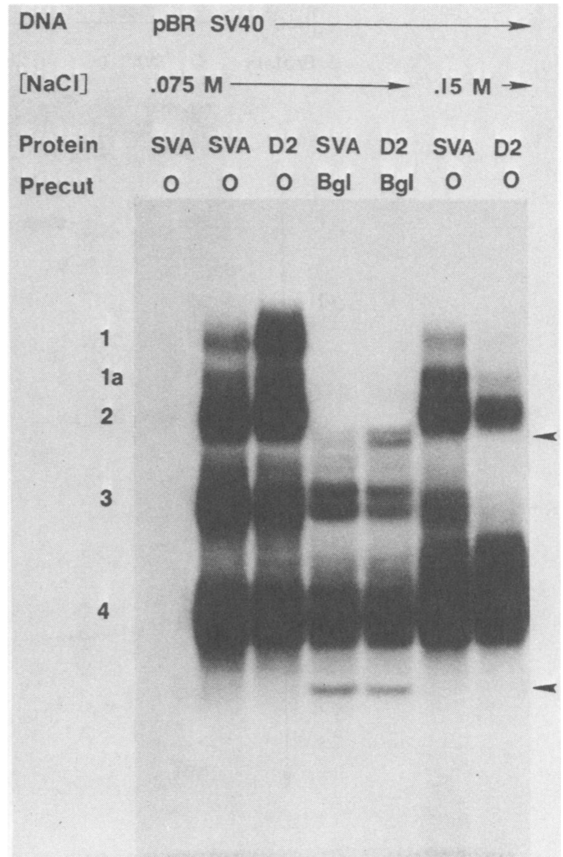

FIG. 2. Mapping of origin DNA fragments protected from DNase by wild-type SV40 A (SVA) or D2 protein. Either nick-translated pBR322 or SV40 DNA (2 ng) was mixed with protein as described in the legend to Fig. 1. DNA binding, DNase treatment, and analysis of DNA fragments are described in the text. The nick-translated DNA in two of the wells was precut with *BglI* before the fragment protection assay was performed. The arrows indicate new fragments of DNA protected after precutting DNA with *BglI*.

DNase footprinting assay. DNase footprinting (5) has been used to define the limits of protein bound to DNA. Unlike the conditions of the fragment assay where nonprotected DNA is totally hydrolyzed, each DNA molecule is cut once in the footprint assay. When run on a sequencing gel and autoradiographed, the end-labeled fragments present a ladder of cut DNA

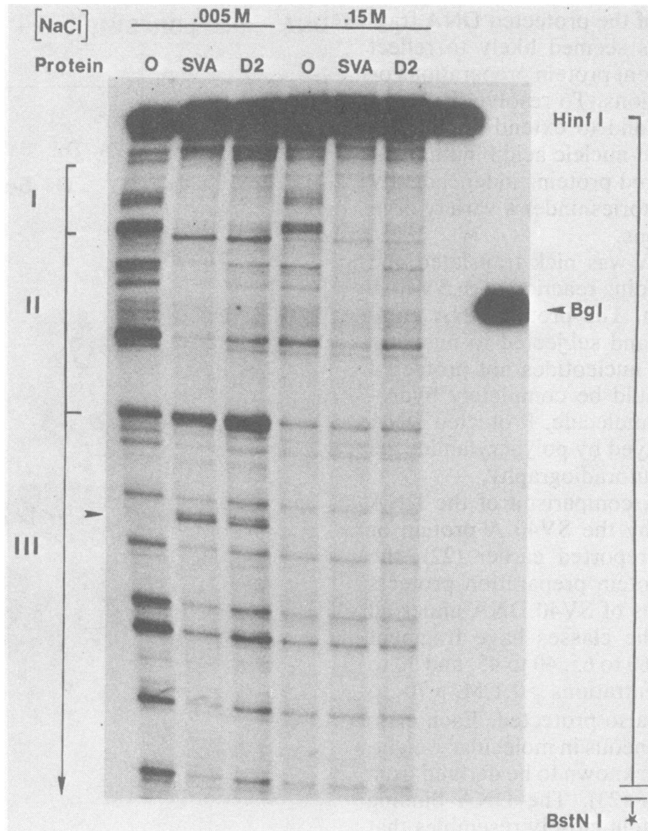

FIG. 3. DNase footprint of SV40 origin DNA protected by SV40 A (SVA) or D2 protein. The *Bst*NI-*Hin*fI fragment was radiolabeled on the 3′ late end of the early strand. DNA binding, DNase digestion, and DNA processing are described in the text. Protected regions I, II, and III are outlined by brackets on the left. A distinct hypersensitive site in region III is shown by the arrow. The last two wells show control untreated and *Bgl*I-digested origin fragments as indicated by the bracket and the arrow on the right.

ideally differing by a single nucleotide. When a region of DNA is protected from DNase by bound protein, a gap appears in the ladder of randomly cut fragments and defines the limits of DNase protection. For these experiments, the double-stranded *Bst*NI-*Hin*fI restriction fragment containing the SV40 origin of replication was isolated and ^{32}P labeled at the 3′ late end of the early strand (see Materials and Methods). DNA binding was examined under conditions with high and low concentrations of NaCl.

The results shown in Fig. 3 confirm that both proteins protect three similar regions of origin DNA under the in vitro binding conditions examined here. Region II maps from nucleotide 5,212 to nucleotide 35 on the early strand (BBB numbering system; 29). The outer limits of regions I and III are more difficult to define precisely, but they extend approximately 35 to 40 and 85 to 90 bp to the early and late sides of region II, respectively. Region III also has a distinctive hypersensitive site that is evident only at 0.005 M NaCl. The relationship of the footprint regions to the map locations of DNA fragments detected by the fragment assay (Fig. 1) is discussed in the accompanying study (23). The results from both assays strongly suggest that the wild-type A protein and the D2 hybrid protein recognize and protect the same regions of DNA. However, DNA binding by D2 protein is more sensitive to the high ionic concentrations.

DMS footprint assay. To identify protein-DNA contact areas, we used a DMS footprinting assay. An SV40 DNA fragment containing the origin of replication was radioactively 3′-end labeled on either the early end or the late end as described in Materials and Methods. Following the methodology of Gilbert et al. (6), guanine residues of the DNA were reacted with DMS in the presence or absence of SV40 A protein or D2 protein. After the methylation reaction and

DNA cleavage, the fragments were displayed by urea-polyacrylamide gel electrophoresis and autoradiography.

Figures 4 and 5 show the location of guanines with altered methylation in the protein-DNA complex. The position of each guanine residue is indicated next to the gels. Guanines are shown on the same line when they occur at adjacent positions in the sequence. Circles indicate hyporeactive residues, and dots indicate hyperreactivity. The brackets in the figures denote binding regions I, II, and III as determined by DNase footprinting (23). In each region, the

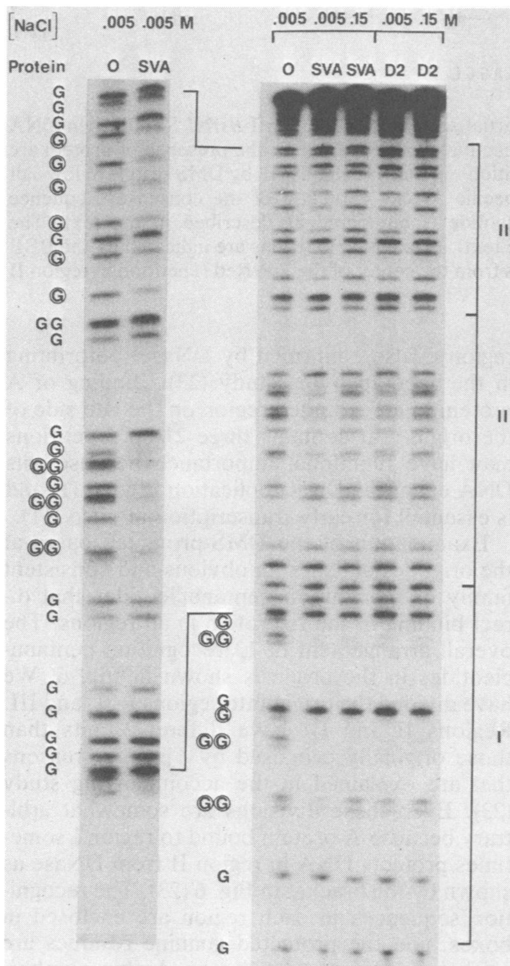

FIG. 4. DMS footprint of SV40 origin DNA protected by SV40 A (SVA) or D2 protein. the *BstNI-HinfI* fragment was radiolabeled on the 3' early end of the late strand. DNA binding, DMS reaction, and DNA processing are described in the text. Guanine residues in the fragment sequence are depicted with the letter G. Protected guanines are circled. Regions of DNA protected in the DNase footprint assay are outlined as I, II, and III (23).

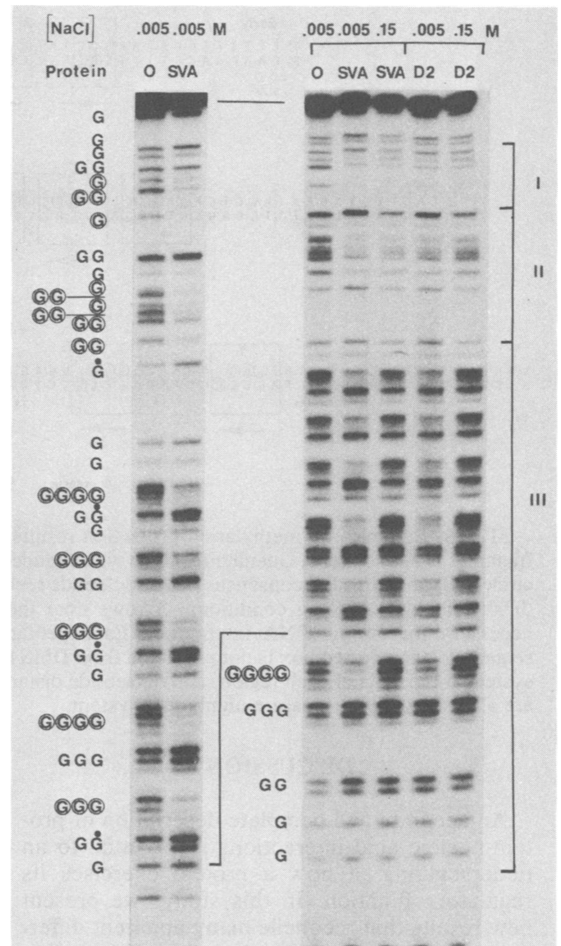

FIG. 5. DMS footprint of SV40 origin DNA protected by SV40 A (SVA) or D2 protein. The *BstNI-HinfI* fragment was radiolabeled on the 3' late end of the early strand. DNA binding, DMS reaction, and DNA processing are described in the text. The guanine residues in the fragment sequence are depicted with the letter G. Protected guanines having a reduced reactivity towards DMS when protein is present are circled. Those guanines which become hyperreactive towards methylation are marked with a dot.

SV40 A protein protects multiple sets of guanines. In general, the guanines most completely protected against methylation are in region I and those least protected are in region III. The most protected bases are also the most resistant to the effect of 0.15 M NaCl on binding. D2 protein establishes a similar protection pattern. In the DMS protection assay, as in the other assays, it was found that D2 protein binds origin DNA with somewhat less efficiency than does SV40 A protein. This result is consistent with an earlier observation noting that a wild-type gene *A* product purified from HeLa cells was able to bind with greater efficiency than D2 protein (11).

Early

I

5' CTTTTTGCAAAAGCCTAGGCCTCCAAAAAAGCCTCCTCA
3' GAAAAACGTTTTCGGATCCGGAGGTTTTTTCGGAGGAGT

5174 5212

II

CTACTTCTGGAATAGCTCAGAGGCCGAGGCGGCCTCGGCCTCTGCATAAATAAAAAAAATTAGT
GATGAAGACCTTATCGAGTCTCCGGCTCCGCCGGAGCCGGAGACGTATTTATTTTTTTTAATCA

5213 33

III

CAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGACTATG
GTCGGTACCCCGCCTCTTACCCGCCTTGACCCGCCTCAATCCCCGCCCTACCCGCCTCAATCCCCGCCCTGATAC

34 108

Late

Consensus: 5' GAGGC 3'
TG

FIG. 6. Summary of methylation protection results. Partial sequence of the *Hin*fI-*Bst*NI SV40 origin DNA fragment is shown here. Guanine residues with reduced reactivity towards DMS in the presence of protein are circled. Boxes surround consensus pentanucleotide recognition sequences established by DMS results at low salt (0.005 M NaCl) binding conditions. Arrows note the specific 5′-to-3′ direction of the consensus sequence necessary for protein-DNA interaction. Each pentanucleotide is numbered as described in the text. The sequence in the dotted box is not protected from DMS (see text). Nucleotide locations are indicated by the BBB system at the ends of each region, and nucleotide distances from the center of the inverted repetition in region II are also shown by a separate numbering system.

DISCUSSION

An accurate and complete description of protein-nucleic acid interactions is essential to an understanding of how a protein exercises its regulatory function. In this study, we present new results that reconcile many apparent differences in previous reports describing the binding of SV40 A protein to the origin of replication. In essence, the variations reflect quantitative differences in binding affinities at different salt concentrations rather than qualitative differences in binding patterns. The salt sensitivity is exaggerated in the case of the D2 hybrid protein that was used in the earliest binding studies. Rather than dwell on the details of past discrepancies, we will use results accumulated under a spectrum of conditions to provide a more complete description of binding at the origin of replication.

DMS protection at 0.005 M NaCl demonstrates contact with the A protein over a broad span of DNA (Fig. 6). The early limit of this space is in good agreement with previous studies by Tjian (27), but the late limit extends well beyond boundaries established by the same method at 0.15 M NaCl. The new limit is consistent with an earlier study that used an exonuclease assay under low salt conditions, although the exonuclease assay could not be used to define the internal arrangement of protein in the adjacent region (16). The overall span of the origin region is also confirmed by DNase footprinting in the accompanying study (23). Binding of A protein to an extended region on the late side of the origin that contains three 21-bp repetitions may have functional importance because this DNA enhances DNA replication in vivo (1) and is essential for early transcription in vitro (11).

Examination of the DMS-protected bases at the origin establishes an obvious and consistent family of recognition pentanucleotides that direct binding of the A protein in all regions. The overall arrangement of 13 recognition pentanucleotides in the origin is shown in Fig. 6. We have divided the origin into regions I, II, and III. Regions II and III have different limits than those originally proposed by Tjian for reasons that are explained in the accompanying study (23). Even these divisions are somewhat arbitrary because A protein bound to region I sometimes protects DNA in region II from DNase as shown by the bracket in Fig. 6 (23). The recognition sequences in each region are enclosed in boxes, and the protected guanine residues are circled. Each box is given an Arabic number, and we propose an abbreviated nomenclature to identify recognition sequences wherever they may occur. The pentanucleotide is represented by a capital P that is followed by a Roman numeral designating the region of its location and by an Arabic numeral to indicate each recognition sequence appearing in an early to late direction in that region.

Table 1 arranges the 13 pentanucleotides in a hierarchy of binding affinities. The hierarchy was established by titrating the amount of NaCl required to suppress each site and by determining the amount of protein needed to protect each site (data not shown). The hierarchy of binding of individual pentanucleotides conforms to the general hierarchy of binding of the surrounding region with one exception. PI-1 binds protein well after the other pentanucleotides in regions I and II. In some cases equivlent pentanucleotides differ in the hierarchy of binding. Hence, adjacent sequences also determine binding affinities. A few guanine residues outside the pentanucleotides are also incompletely protected from DMS, but in every case these occur as a single nucleotide between two contact pentanucleotides. The consensus sequence of the pentanucleotide is shown at the bottom of Table 1. The sequence agrees with the previous identification by Tjian of the ideal pentanucleotide found in regions I and II (27) but has now been extended to account for all contact areas. The same sequence with the opposite polarity occurs several times within the origin but is not protected from DMS. Only a single pentanucleotide with the proper polarity is apparently not used for protein contact (shown with dotted lines in Fig. 6). In this case, the unused pentanucleotide overlaps both PI-1 and PI-2. We presume that the 5′-GAGGC-3′ sequence in PI-2 precludes binding to the overlapping and less favorable 5′-TAGGC-3′ sequence. A number of experimental findings support this idea. Under conditions of high NaCl and low protein concentration, only the guanines in PI-2 are protected from DMS (data not shown). Under conditions of low NaCl and high protein concentration, all guanines in both PI-1 and PI-2 are completely protected, whereas the guanine tightly packed between these pentanucleotides is only partly protected. A similar partial protection of guanines occurs between adjacent pentanucleotides in region II where no pentanucleotides overlap. Hence, we conclude that binding to PI-1 and PI-2 accounts for the protection of all guanines in the overlapping area.

The arrangement of pentanucleotides is fundamentally different in regions I, II, and III. Figure 6 emphasizes the orientation of each recognition sequence with arrows. In region I, three pentanucleotides arranged as direct repetitions extend over 23 bp. In region II, four contact sequences also span 23 bp, but they are arranged as pairs of inverted repetitions. In region III, six pentanucleotides aligned in the same direction span 59 bp. These fundamental differences in DNA arrangement are likely to determine different patterns of protein binding within each region. If the simplest unit of protein recognition consists of more than a single pentanucleotide, many protein conformations or assembly states would be required to accommodate the diversity of pentanucleotide arrangements in the origin. If a single contact pentanucleotide is the basic unit of protein recognition, then a monomer of A protein could react with each.

TABLE 1. Hierarchy of SV40 A protein contact pentanucleotides at the origin of DNA replication[a]

Location	Sequence				
PI					
2	5′	G	GAGGC	C	3′
3		G	GAGGC	T	
PII					
1		A	GAGGC	C	
2		C	GAGGC	G	
3		C	GAGGC	C	
4		A	GAGGC	C	
PI					
1		C	TAGGC	T	
PIII					
1		T	GGGGC	G	
2		A	TGGGC	G	
3		C	TGGGC	G	
4		A	GGGGC	G	
5		A	TGGGC	G	
6		A	GGGGC	G	

[a] The consensus sequence is 5′-G>T, A>G, G, G, C-3′. The hierarchy within groups has not yet been established.

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LITERATURE CITED

1. **Bergsma, D. J., D. M. Olive, S. W. Hartzell, and K. N. Subramanian.** 1982. Territorial limits and functional anatomy of the simian virus 40 replication origin. Proc. Natl. Acad. Sci. U.S.A. **79**:381–385.
2. **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.
3. **Chou, J. Y., and R. G. Martin.** 1974. Complementation of simian virus 40 mutants. J. Virol. **13**:1101–1109.
4. **DiMaio, D., and D. Nathans.** 1980. Cold-sensitive regulatory mutants of simian virus 40. J. Mol. Biol. **140**:129–142.
5. **Galas, D. J., and A. Schmitz.** 1978. DNase footprinting: a simple method for the detection of protein-DNA binding specificity. Nucleic Acids Res. **5**:3157–3170.
6. **Gilbert, W., A. Maxam, and A. Mirzabekov.** 1976. Contacts between the *lac* repressor and DNA revealed by methylation, p. 139–148. *In* N. Kjeldgaard and O. Maale (ed.), Alfred Benzon Symposium, vol. 9. Academic Press, Inc., New York.
7. **Hand, R.** 1981. Functions of T antigens of SV40 and polyomavirus. Biochim. Biophys. Acta **651**:1–24.
8. **Hansen, U., D. G. Tenen, D. M. Livingston, and P. A. Sharp.** 1981. T antigen repression of SV40 early transcription from two promoters. Cell **27**:603–612.
9. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-

labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
10. **McKay, R., and D. DiMaio.** 1981. Binding of an SV40 T antigen-related protein to the DNA of SV40 regulatory mutants. Nature (London) **289**:810–813.
11. **Myers, R. M., D. C. Rio, A. K. Robbins, and R. Tjian.** 1981. SV40 gene expression is modulated by the cooperative binding of T antigen to DNA. Cell **25**:373–384.
12. **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. U.S.A. **77**:6491–6495.
13. **Reed, S. I., G. R. Stark, and J. C. Alwine.** 1976. Autoregulation of simian virus 40 gene A by T antigen. Proc. Natl. Acad. Sci. U.S.A. **73**:3083–3087.
14. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
15. **Rio, D., A. Robbins, R. Myers, and R. Tjian.** 1980. Regulation of simian virus 40 early transcription *in vitro* by a purified tumor antigen. Proc. Natl. Acad. Sci. U.S.A. **77**:5706–5710.
16. **Shalloway, D., T. Kleinberger, and D. M. Livingston.** 1980. Mapping of SV40 DNA replication origin region binding sites for the SV40 T antigen by protection against exonuclease III digestion. Cell **20**:411–422.
17. **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. U.S.A. **76**:6128–6131.
18. **Shortle, D., and D. Nathans.** 1979. Regulatory mutants of simian virus 40: constructed mutants with base substitutions at the origin of DNA replication. J. Mol. Biol. **131**:801–817.
19. **Spillman, T., D. Giacherio, and L. P. Hager.** 1979. Single strand DNA binding of simian virus 40 tumor antigen. J. Biol. Chem. **254**:3100–3104.
20. **Tegtmeyer, P.** 1972. Simian virus 40 deoxyribonucleic acid synthesis: the viral replicon. J. Virol. **10**:591–598.
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. Andersen, S. B. Shaw, and V. G. Wilson.** 1981. Alternative interactions of the SV40 A protein with DNA. Virology **115**:75–87.
23. **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.
24. **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.
25. **Tenen, D. G., L. L. Haines, and D. M. Livingston.** 1982. Binding of an analog of the simian virus 40 T antigen to wild-type and mutant viral replication origins. J. Mol. Biol. **157**:473–492.
26. **Tjian, R.** 1978. The binding site on SV40 DNA for a T antigen-related protein. Cell **13**:165–179.
27. **Tjian, R.** 1978. Protein-DNA interactions at the origin of simian virus 40 DNA replication. Cold Spring Harbor Symp. Quant. Biol. **43**:655–662.
28. **Tjian, R.** 1981. Regulation of viral transcription and DNA replication by the SV40 large T antigen. Curr. Top. Microbiol. Immunol. **93**:5–24.
29. **Tooze, J.** 1980. DNA tumor viruses, p. 125–204. *In* J. Tooze (ed.), Molecular biology of tumor viruses, 2nd ed., part 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **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.