Identification of a DNA Binding Domain in Simian Virus 40 Capsid Proteins Vp2 and Vp3*

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We have identified both biochemically and genetically a protein domain within the simian virus 40 virion protein Vp3, and within Vp2 since its carboxylic two-thirds are identical to the full-length Vp3, that binds DNA in a sequence nonspecific manner. Both the Vp2 and Vp3 (Vp2/3) components of SV40 and mutant SV40* bound either SV40 or pBR322 DNA equally well. Wild type and mutant Vp2/3 proteins, expressed as fusion proteins with glutathione S-transferase (GST), were tested for their ability to bind DNA. GST-Vp3 bound DNA at physiological salt concentrations with an apparent Kd of 2.5 × 10⁻⁸ M and also bound RNA with 4-fold higher affinity. Over 90% of the nucleic acid binding, and all of the activity, was lost upon removal of the carboxy-terminal 13 and 35 residues, respectively. The DNA binding domain was shown to be distinct and separable from the Vp2/3 nuclear transport signal since mutations within the nuclear transport signal that reduce or abolish nuclear localization of Vp2/3 had no effect on the DNA binding activity of mutant Vp2/3 fusion proteins. The carboxy-terminal 40 residues of Vp2/3 in the form of a β-galactosidase fusion protein, F6, are sufficient for DNA binding and may cause compaction of the DNA. The significance of this DNA binding and possible compaction are discussed in relation to the assembly of virion particles.

The papovaviruses, including murine polyoma virus and simian virus 40 (SV40), areicosahedrally symmetric viruses whose capsids are composed of 72 pentamers of the major structural protein Vp1 (1–3). The mature virion also contains the viral-encoded proteins Vp2 and Vp3, and histones H2A, H2B, H3, and H4 (4). Based on the measurements of the molar ratio of Vp1, Vp2, and Vp3 in virions, there are about 21 Vp2 and 56 Vp3 molecules present in a particle (5). A reasonable assumption then would be that each of the 72 Vp1 pentamers interacts with either one Vp2 or one Vp3 molecule. Vp2 contains all of the amino acids of Vp3 plus an additional 118 amino-terminal residues and as such, the interaction of Vp2 and Vp3 with the Vp1 pentamers could be the same. The electron density map of polyoma virions at 25 Å resolution has shown the interior as a mine-shaped structure with 72 prongs, each of which has been proposed to be one molecule of either Vp2 or Vp3, each radiating from the central minichromosome core into the axial cavities of the Vp1 pentamers (6). The interior structure resolved by the x-ray diffraction data is in agreement with that obtained by electron cryotomy of SV40 particles (1). At the core of the mine-shaped structure, the Vp2 and Vp3 molecules are predicted to interact with the SV40 minichromosome, which consists of a viral, covalently closed circular DNA of 5243 bp and about 21 histone octamers (7–9). In housing the interior structure, the Vp1 pentamer is expected to interact with Vp2 and Vp3 as well as with the minichromosome. SV40 Vp1 has been shown to bind DNA (10), and a sequence nonspecific DNA binding domain in polyoma Vp1 also has been identified within its first 7 amino acids (11). Furthermore, a Vp1-interactive domain has been identified in Vp3 of SV40 (12).

Several lines of evidence suggest that Vp3 interacts with the viral minichromosome and DNA. In addition to its proximity to the viral DNA in the virion particle, Vp3 has been shown to remain complexed with the minichromosome upon dissociation of virions (13, 14). Histone H1, which is absent in the mature SV40, has been reported to be present in the nuclear form of the SV40 minichromosome (15, 16). Thus it has been suggested that one role of Vp3 is to replace H1 and condense the minichromosome during virion packaging (16). A protein domain(s) within SV40 Vp2/3 could function in DNA binding and compaction during virion formation, as has been suggested (13, 15, 17).

The carboxy-terminal 40 residues (195–234 of Vp3) of SV40 Vp2/3 contain multiple domains involved in virion morphogenesis. The Vp2/3 nuclear transport signal (NTS) has been localized to 9 residues (198–206 of Vp3) and has been shown to be necessary and sufficient for nuclear targeting of both the Vp3 protein and non-nuclear carrier proteins (18, 19). Residues 222–234 of Vp3 specify a Vp1-interactive determinant of Vp2/3: fusion of the last 40 residues of Vp3 to β-galactosidase promotes association with Vp1, while deletion of the last 13 residues abolishes the interaction between Vp3 and Vp1 (12, 18). Additionally, the last 40 residues are rich in basic residues, and several stretches of amino acid sequence similarity to histones H2A, H2B, and H1 are found within the carboxyl portion of Vp2/3 (17). In this report we have examined the interaction of SV40 Vp3 with DNA.

MATERIALS AND METHODS

Plasmid Constructions—Plasmids coding for glutathione S-transferase (GST)-Vp3 fusion proteins were constructed by subcloning 0.95 kilobase (kb) EcoRIIII to EcoRII restriction fragments of SV40 DNA (SV40 nucleotides 172–1793) into the Smal/EcoRI-digested plasmid pGEX-3X, which contains the coding sequence for GST from...
Mapping of SV40 Vp2/3 DNA Binding Domain

Schistosoma japonicum under control of the inducible tac promoter (20; Pharmacia LKB Biotechnology Inc.). References to SV40 nucleotide numbers are in the SV system (4). DNA manipulations were performed as described (21). All of the GST fusion proteins contain 27 unique Vp2 residues (Vp2 residues 92–118) upstream of the Vp3 initiation codon in Vp3. The plasmid pGEX-Vp3AC35 was created by subcloning the Eco47III to the EcoRI fragment derived from the plasmid pSV23A (17) into pGEX-3X. The plasmid pGEX-Vp3AC35 was made by replacing the AorII (SV40 nucleotide 1078) to EcoRI restriction fragment from pGEX-Vp3Ac25 with its counterpart fragment (AorII to EcoRI) derived from the plasmid pSp6Vp3Ac13 (18). In the mutant Vp3AC5, the carboxy-terminal 35 residues of Vp3 were deleted and the protein contained an additional 3 residues (LTD) at the end. In the mutant Vp3Ac13, the last 13 residues of Vp3 were deleted and the protein acquired 6 additional residues (LTDNE5) at the end.

The plasmids B290E1, B290F6, and B290F6 replacement were described (12, 19). All constructions were verified by restriction enzyme analysis and by dideoxynucleotide, double-stranded, plasmid sequencing (22, 23), as well as by their ability to produce anti-Vp3 immunoreactive species with the expected molecular weights.

Antibodies and Immunoblotting—Affinity-purified anti-Vp3 IgG was prepared from polyclonal rabbit anti-Vp3 sera (24) using SV40 virion-derived Vp3 immobilized on nitrocellulose blots as described (25). Immunoblotting was performed as described (5). The transcription vectors pSp6Vp1 and pSp6Vp3 (12) were linearized with SstI to make SV40-derived transcripts and pSp64 (31) was linearized with Ddel to generate a transcript of comparable size. After RNA synthesis, the DNA was digested with Dnal, and unincorporated nucleotides were removed by precipitation with ethanol in the presence of ammonium acetate (21).

Detection of DNA Binding Proteins on Nitrocellulose—Southwestern assays, after SDS-PAGE (32) and electrophoresis of proteins to nitrocellulose, replicas were blocked in buffer A (10 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, pH 7.5) containing 1 × Denhardt solution for at least 30 min at 22 °C (23). Nick-translated, [32P]-radiolabeled pBR322 or SV40 DNAs (specific activity: 106 cpm/μg) were incubated with blots in sealed bags in the above buffer (10 ng/ml in 5 ml) with or without 10 mM DTT for 1 h at 22 °C. The blots were then washed twice for 5 min each in 50 ml of buffer A, twice for 15 min in 100 ml, and twice for 30 min in 100 ml, at 22 °C before being air-dried and exposed to film for autoradiography.

Radiolabeling of DNA and RNA—DNAs were nick-translated using [α-32P]ATP (specific activity: 3000 Ci/mmol; ICN, Irvine, CA) or 5’-end-labeled using T4 polynucleotide kinase (specific activity: 3000 Ci/mmol; ICN) as described (21). The 179- and 1541 bp fragments were derived from pSp6Vp1 (12) and the 476-, 1014-, and 5243-bp fragments were derived from SV40 DNA.

Radiolabeled RNAs were prepared by in vitro transcription using SP6 polymerase and [α-32P]GTP (specific activity: 450 Ci/mmol; ICN, Irvine, CA). The transcription vectors pSp6Vp1 and pSp6Vp3 (12) were linearized with SstI to make SV40-derived transcripts and pSp64 (31) was linearized with Ddel to generate a transcript of comparable size. After RNA synthesis, the DNA was digested with DNase I, and unincorporated nucleotides were removed by precipitation with ethanol in the presence of ammonium acetate (21).

Gel Shift DNA Binding Assays—Varying amounts of proteins were incubated with 2 ng of [32P]-labeled DNA fragments (5000 cpm) for 30 min at 22 °C in 15 μl of buffer B (20 mM Tris-HCl, pH 7.5, 20 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT) containing 50 mM NaCl. The reactions were run on one-sixth the mass, judging from SDS-PAGE and Coomassie Blue staining and from immunoblots using monoclonal anti-β-galactosidase IgG.

Filter Binding Assays—Filter binding assays were performed in duplicate using affinity-purified GST-Vp3 and radiolabeled probe (1 ng; specific activity: 5 × 106 cpm/μg) in buffer A as described (34). For competition experiments, the indicated amounts of competitor nucleic acids were added to buffer A containing 0.16 μM GST-Vp3 immediately before addition of the radioactive probe. Background binding of the probe to the filters was corrected for by performing identical reactions in the absence of GST-Vp3 and typically accounted for less than 8% of the maximum counts bound. Nonradioactive SP64, Vp1, and Vp3 RNAs were prepared by in vitro transcription using Ddel-linearized pSP64, SstI-linearized pSp6Vp1, and pSp6Vp3, and quantitated spectrophotometrically as described (21). Single-stranded M13 and yJ14 DNA were from Life Technologies Inc.-Bethesda Research Laboratories.

Denaturation—Denaturation was performed on autoradiograms, within the linear response range of the film, using a Bio-Rad model 620 video densitometer.
RESULTS

DNA Binding by Virion-associated Vp3

We have purified wild type virions, as well as mutant virions containing a single amino acid change in the Vp2- and Vp3-NTS, Vp3 lysine 202 to threonine (SV40mut), and tested their proteins (Fig. 1A, lanes 2 and 3) for their ability to bind nick-translated 32P-labeled SV40 DNA in a Southwestern assay (Fig. 1B). All of the virion proteins, Vp1, Vp2, and Vp3 of both wild type (Fig. 1B, lane 2) and SV40mut (Fig. 1B, lane 3) bound DNA, while non-virion proteins, e.g., molecular weight markers, did not (Fig. 1B, lane 1). Virion-associated histones also bound DNA (Fig. 1B). The source of labeled DNA did not appear to affect binding. Nick-translated pBR322 plasmid DNA was also used and gave the same results as SV40 DNA (data not shown). These results suggest that Vp1, Vp2, and Vp3 contain DNA binding domains and that the lysine at residue 202 of Vp3 (and the corresponding residue of Vp2, referred to collectively as Vp2/3) is not essential for this binding activity. Bacterially expressed polyomavirus Vp1 has been shown to bind DNA, but only when DTT is absent from the binding buffer (11). We tested if this was also the case for the SV40 protein, and found that the presence or absence of DTT did not effect the level of DNA binding by any of the SV40 proteins (data not shown).

DNA Binding by GST-Vp3 Fusion Proteins

Southwestern Analysis—In order to identify the domain(s) of Vp3 responsible for this Vp3 DNA binding activity, full-length Vp3 and two Vp3 carboxyl-terminal deletions were expressed as fusion proteins with GST. The fusion proteins, from the insoluble fraction of lysed cells, were separated by SDS-PAGE, visualized by Coomassie Blue staining (Fig. 2A), and transferred to nitrocellulose (Fig. 2, B and C). When reacted with affinity-purified rabbit anti-Vp3 IgG followed by 125I-labeled protein A (Fig. 2B), one major and four minor anti-Vp3 reacting bands were present (Fig. 2B, lane 1). The major anti-Vp3 reacting band corresponded to the major band seen by mass (compare Fig. 2, B, lane 1, with A, lane 1), and corresponds to the expected size of the GST-Vp3 (53–55 kDa). The source of the minor immunoreactive bands is unknown but we believe that the faster migrating bands are degraded fusion proteins.

When the proteins, after transfer to nitrocellulose, were incubated with 32P-labeled DNA, the wild type GST-Vp3 bound the labeled DNA (Fig. 2C, lane 1). The major immunoreactive band as well as 2 of the faster migrating GST-Vp3 related bands bound DNA, while none of the other proteins present on the blot reacted with the DNA. DNA binding by GST-Vp3 was abolished in 0.3 M NaCl, but was still detectable in 0.2 M NaCl and was strongest in 0.05 M NaCl (data not shown).

To localize the Vp3 DNA binding domain, we constructed two carboxyl-terminal deletion mutants of Vp3, since the last 40 residues show a high degree of sequence similarity to regions of histones H1, H2A, and H2B (17). Two deletion mutant fusion proteins which removed either the last 13 (GST-Vp3AC13; Fig. 2, A and B, lane 2) or 35 (GST-Vp3AC35; Fig. 2, A and B, lane 3) residues of Vp3 failed to bind DNA by this assay (Fig. 2C, lanes 2 and 3, respectively). However, residual DNA binding activity of GST-Vp3AC13 was demonstrated with protein samples that had not undergone SDS denaturation. Using a dot blot assay, GST-Vp3 reproducibly bound DNA at NaCl concentrations up to 0.3 M (Fig. 2D, lane 1; data not shown). While GST-Vp3AC35 failed to bind DNA above background levels (Fig. 2D, lane 3), faint DNA binding by GST-Vp3AC13 was detected (Fig. 2D, lane 2). Densitometry of autoradiograms revealed that GST-Vp3AC13 bound 6.7 ± 0.3% of wild type GST-Vp3 levels, based on data from three experiments (data not shown).

Separation of the Vp3 DNA Binding Domain and the Nuclear Transport Signal—Since the mutant Vp3mut bound DNA to the same extent as the wild type Vp3 (Fig. 1), the Vp2/3-NTS (residues 198–206: GPRKKKAKKL), which is highly basic and includes the 202 lysine, appears not to be a part of the DNA binding domain. To confirm that the NTS and the Vp3 DNA binding domain are independent of each other, we tested the DNA binding activity of 5 mutant proteins, whose single or double point mutations are within the Vp2/3-NTS. These mutations in GST-Vp3mut, GST-Vp3AC13, GST-Vp3AC35, GST-Vp3202T, GST-Vp3202K, and GST-Vp3202N, change either Vp3 lysine 202 and/or arginine 204, both of which affect the function of this nuclear targeting domain (19, 44). All 5 mutant proteins reacted with anti-Vp3 IgG (Fig. 2B, lanes 4–8) and bound 32P-labeled DNA by Southwestern and dot blot assays (Fig. 2C, lanes 4–8 and D, lanes 4–8). These results indicate that the Vp2/3-NTS is functionally independent of the DNA binding domain, since mutations which are deleterious to Vp3 nuclear translocation did not greatly affect its DNA binding ability.

DNA Binding by GST-Vp3 Fusion Proteins in Solution—Since the GST-Vp3 fusion proteins appeared to bind more favorably with DNA when assayed under nondenaturing conditions (Fig. 2D), we examined the interaction of DNA with the fusion protein in solution using a DNA fragment mobility shift assay. Fig. 3 shows the result of a typical experiment. When affinity-purified GST-Vp3 was added in increasing amounts to a 179-bp end-labeled DNA fragment, the protein-free DNA band (Fig. 3A, lane 1, arrowhead) disappeared and slower migrating bands appeared (Fig. 3A, lanes 2 and 3). As was seen with the Southwestern assays, neither of the truncated proteins, GST-Vp3AC13 or GST-Vp3AC35, bound the DNA fragment, and the protein-free DNA remained at its position with equivalent band intensity (Fig. 3A, lanes 4–7). The mobility shift assay was also performed with a longer DNA fragment (1541 bp). The results were similar to those for the 179-bp fragment: GST-Vp3 was able to retad the...
Fig. 2. Western and Southwestern analyses of GST-Vp3 fusion proteins. Approximately 4 μg of each of the urea-solubilized GST-Vp3 fusion proteins (8–15 μg of total protein) were separated per lane on 12.5% SDS-polyacrylamide gels and Coomassie Blue stained (A), or transferred to nitrocellulose and probed with 0.4 μg/ml affinity-purified anti-Vp3 IgG followed by 125I-labeled protein A (B), or probed with nick-translated 32P-labeled DNA in buffer A containing 1 × Denhardt solution without DTT (C). For dot blot analysis (D), 0.1 μg of each of the fusion proteins was blotted directly onto nitrocellulose and probed with 32P-labeled DNA as in C. The samples are: GST-Vp3 (lane 1), GST-Vp3ΔC13 (lane 2), GST-Vp3ΔC35 (lane 3), GST-Vp3ΔC35 (lane 4), GST-Vp3ΔC35 (lane 5), GST-Vp3ΔC35 (lane 6), GST-Vp3ΔC35 (lane 7), and GST-Vp3ΔC35 (lane 8). Positions of molecular weight standards, phosphorylase b (97 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), and carbonic anhydrase (29 kDa) are indicated, from the top to the bottom, respectively, to the left of A.

Fig. 3. Binding of GST-Vp3 fusion proteins to DNA in solution. A, affinity-purified GST-Vp3 (lanes 2 and 3), GST-Vp3ΔC13 (lanes 4 and 5), and GST-Vp3ΔC35 (lanes 6 and 7) were incubated with a 179-bp 32P-end-labeled DNA fragment and separated by gel electrophoresis as described under “Materials and Methods.” The amount of fusion protein added to each reaction is indicated at the top of the figure. DNA in the absence of protein is shown in lane 1. B, a 1541-bp 32P-end-labeled DNA fragment was incubated in the absence (lane 1) or presence (lane 2) of GST-Vp3.

DNA fragment (Fig. 3B, compare lanes 1 and 2). The addition of a 15- or 1500-fold molar excess of unlabeled sheared salmon sperm DNA to the reaction mixture together with the 179-bp 32P-labeled DNA fragment abolished the mobility shift (data not shown). Thus the interaction of GST-Vp3 with DNA is sequence nonspecific, confirming the result presented above.

The mobility shift remained unaffected until the salt concentration was raised to 0.5 M and could still be detected at 1.25 M NaCl but not at 1.5 M NaCl (data not shown). That the interaction between GST-Vp3 and DNA is relatively stable at high salt concentrations was confirmed by independent experiments. When 32P-labeled DNA was incubated with or without GST-Vp3 at various NaCl concentrations and separated from protein-free DNA by gel filtration, DNA binding was detected up to 1 M NaCl (data not shown).

Characterization of Nucleic Acid Binding.—The above results suggest that the interaction of the Vp3 DNA binding domain with DNA is sequence nonspecific. We thus used a filter binding assay to examine the extent of nucleic acid binding to GST-Vp3. GST-Vp3 bound to SV40 and pBR322 DNA with identical apparent Kd values of 2.5 × 10^-9 M (Fig. 4A). In competition experiments using unlabeled nucleic acids to compete for the binding of 32P-labeled SV40 DNA (Fig. 4B), unlabeled SV40 DNA, pBR322 DNA, and tRNA all showed the same degree of competition, with a competitor to probe ratio of 80 to 1 (w/w) giving 50% inhibition. In contrast, single-stranded DNA, and both SV40-derived transcripts and SP6 transcripts inhibited DNA binding at ratios that were about 30-fold lower than those of unlabeled duplex DNA (Fig. 4B). Thus GST-Vp3 bound slightly better to RNA and single-stranded DNA than to double-stranded DNA. This was corroborated by filter binding assays using 32P-labeled RNA: GST-Vp3 bound to viral and nonviral RNAs equally well with an apparent Kd of 6.6 × 10^-9 M (Fig. 4A).

Using the same point mutants and truncated GST-Vp3 fusion proteins as for the DNA binding experiments, we probed proteins immobilized on nitrocellulose with 32P-labeled RNA. Wild type Vp3, as well as all of the NTS point mutants, bound RNA, while GST-Vp3ΔC13 and GST-Vp3ΔC35 did not bind RNA (not shown). Thus the same domain that is required for DNA binding is also responsible for RNA binding. We also performed a Northwestern blot using virion-derived Vp3 and found that it too was able to bind RNA (not shown). Thus the last 35 residues of Vp3 constitutes a general nucleic acid binding domain that shows no sequence specificity for either DNA or RNA.

DNA Binding Activity of the Vp3 Carboxyl 40 Residues

Interaction of Vp3-β-Galactosidase Fusion Proteins with a 179-bp DNA Fragment.—The sequence nonspecific DNA binding activity of the carboxyl-terminal 40 residues of Vp3 was next tested. Three β-galactosidase-Vp3 fusion proteins were used (19): two proteins, F6 and F6202-17, contained the carboxyl-terminal 40 residues of Vp3 and its mutant Vp3ΔC35...
was used in gel shift experiments and behaved similarly to DNA binding domains (data not shown).

F6, again demonstrating the separation of the NTS from the protein molecule binds one DNA molecule, we estimated the $K_d$. Half of the input DNA remained as protein-free DNA when 1.35 µg of F6 was added to the reaction mixture, giving an apparent $K_d$ value of $2 \times 10^{-7}$ M.

**Binding of F6 to Long DNA Fragments**—We examined the effect of fragment size on the DNA binding activity of F6. When a 1541-bp 32P-labeled DNA fragment was reacted with F6, band shifts as seen in Fig. 6 were obtained. Instead of the band retardation that was seen when F6 was incubated with the shorter DNA fragment (Fig. 5A, lanes 1–4) or when GST-Vp3 was assayed by the gel shift assay using either short or long DNA fragments (Fig. 3), DNA-F6 protein complexes which migrated with increased mobilities, as compared to protein-free DNA (Fig. 6A, lane 1, arrowhead), could be seen (Fig. 6A, lanes 2–4, open and closed circles). When the amount of the F6 fusion protein was raised to 4 µg, a third species was seen which migrated in the gel with a reduced mobility (Fig. 6A, lane 4, diamond). These three discrete protein-DNA species migrated with the apparent mobilities of 0.4-, 0.85-, and 2.8-kb DNA. β-Galactosidase, purified by the same procedure as F6, did not cause a mobility shift of the 1541-bp fragment, indicating that the F6-induced mobility shift was due to the fusion protein and was not due to contaminants in the preparation (Fig. 6B, lanes 8 and 9).

We further tested if these faster migrating bands were caused by degradation of the input DNA, perhaps by a nuclelease that contaminated our preparation. Reactions identical to those in lanes 2–4 of Fig. 6 were treated further, after protein-DNA complexes had been allowed to form, with either proteinase K or SDS prior to electrophoresis. The 1541-bp fragment regained its initial mobility, as protein-free DNA, after the proteinase K treatment (Fig. 6A, lanes 5–7). Treatment of the reaction mixture with 3% SDS prior to electrophoresis had a similar effect (data not shown). We conclude that the input DNA was not being degraded, but that the faster and slower migrating DNA species were distinct complexes between F6 and DNA.

Two other sizes of labeled fragments were tested for their ability to bind F6. A 476-bp fragment behaved similarly to the 179-bp fragment resulting in bands and smears of DNA-protein complexes with decreased mobilities (Table I). When the 1014-bp DNA was used, a faster migrating DNA-protein complex was again observed that had an apparent mobility of 0.85 kb, similar, if not identical to the species seen following the interaction with the 1541-bp DNA (Table I).

Thus, the results using the β-galactosidase-Vp3 fusion proteins demonstrate that the last 40 residues of Vp3 are sufficient to bind DNA, and furthermore, that when present as a multimer, this fusion protein has the ability to perhaps compact long DNA fragments.

**DISCUSSION**

We have shown that Vp3, derived from SV40 virions or produced as a fusion protein in E. coli, is capable of binding duplex DNA as well as single-stranded nucleic acids in a sequence nonspecific manner. We have localized the nucleic acid binding domain to the carboxyl-terminal 40 amino acids of this protein and have shown that the last 13 residues are required for binding. We also have shown that this binding domain is independent from the Vp2/3 NTS. Furthermore, the last 40 residues of Vp3 are sufficient for the binding, and...
FIG. 5. Binding of β-galactosidase–Vp3 fusion proteins with 179-bp DNA fragments. A, 179-bp 32P-end-labeled DNA fragments were incubated with increasing amounts of fusion protein F6 (lanes 1–4) or β-galactosidase (lanes 6–9), electrophoresed on 6% polyacrylamide gels, and subjected to autoradiography. Lanes 5 and 10 show protein-free DNA (arrowhead). The reactions in lanes 1–4 contained 4.8, 2.4, 1.2, and 0.3 µg of F6, respectively, while the reactions in lanes 6–9 contained 16, 8, 4, and 1 µg of β-galactosidase, respectively. B, the 32P-labeled DNA fragments were incubated with 0.5, 1, 4, and 8 µg of fusion protein E1 (lanes 2–5, respectively). Lane 1: protein-free DNA (arrowhead).

FIG. 6. Binding of F6 to 1541-bp DNA fragments. A, lanes 1–4, increasing amounts of F6 were incubated with a 1541-bp 32P-end-labeled DNA fragment and then electrophoresed as described under “Materials and Methods.” Lane 1 shows DNA fragments incubated without F6 (arrowhead) while lanes 2–4 show reactions containing 1, 2, and 4 µg of F6, respectively. The position of protein-free DNA (arrowhead) and discrete DNA-protein complexes, which migrated with decreased or increased mobilities compared to protein-free DNA, are marked to the left of the gel (diamond, open circle, and closed circle). The apparent mobilities of all the discrete DNA species were determined by comparison to known molecular weight markers and are shown to the right (2.8, 1.5, 0.85, and 0.4 kb). Reactions identical to those in lanes 2–4 were further treated with proteinase K before being electrophoresed as above (lanes 5–7). B, a 1541-bp 32P-end-labeled DNA fragment was incubated in the presence (lane 8) or the absence (lane 9) of 8 µg of β-galactosidase, and electrophoresed as in A.

TABLE I

<table>
<thead>
<tr>
<th>Fragment size</th>
<th>Apparent sizes of shifted DNA-protein complexes</th>
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<tr>
<td>179 bp</td>
<td>&gt;0.20 kb</td>
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<tr>
<td>476 bp</td>
<td>&gt;0.50 kb</td>
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<tr>
<td>1014 bp</td>
<td>&gt;1.0 kb</td>
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<tr>
<td>1541 bp</td>
<td>&gt;2.8 kb</td>
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when present as a multimeric form have the ability to compact DNA.

The substitutions of the basic residues in the Vp2/3 NTS by noncharged residues can abolish Vp2/3 nuclear localization (18, 19, 44). These mutant proteins, in the form of a mutant virion Vp2/3, GST fusion proteins, and β-galactosidase fusion proteins, bound DNA equally as well as the wild type Vp3. Thus, we effectively demonstrated that the two domains, the Vp2/3 NTS and the DNA binding domains, are separable. The results obtained using the truncated proteins localized the majority of the DNA binding domain to the carboxy-terminal 13 residues of the protein, downstream of the NTS. However, what contributes to the residual DNA binding activity of the GST-Vp3ΔC13 is unknown. It is likely that the adjacent upstream 22 residues (from 195 to 221) are responsible for the residual activity. However, construction of the pGEX-Vp3ΔC13 plasmid resulted in the addition of 6 amino acids to the carboxyl terminus of the mutant protein, which are not normally present in Vp3, and these additional residues, LTDNSS, could contribute to the residual activity.

Our study identified a Vp2/3-DNA binding domain within the Vp3 coding sequence and 27 residues unique to Vp2 (see “Materials and Methods”), but did not test the activity in the remaining amino-terminal 91 residues of Vp2. How these residues contribute to the interaction with DNA or to overall virion assembly will be our future study. It is interesting to note that polyomavirus Vp2 and Vp3 do not contain a region similar to the carboxy-terminal 28 residues of SV40 Vp2/3 (4). The polyoma proteins terminate immediately after the region similar to the SV40 Vp2/3-NTS (19), and it is not known whether the polyoma proteins bind to DNA. Thus the relative organization of the polyoma minichromosome and Vp2/3 molecules to Vp1 pentamers could be different from that in SV40.

The apparent $K_d$ for DNA of the Vp3 DNA binding domain, determined by filter binding assays using GST-Vp3 was $2.5 \times 10^{-8}$ M. That identical $K_d$ values were obtained using either SV40 DNA or pBR322 DNA confirms that this interaction is sequence nonspecific. The apparent $K_d$ for the last 40 residues of Vp3 expressed as the F6 fusion protein is about $2 \times 10^{-7}$ M. This difference in affinities could be due to the multimeric nature of F6, in which the fused Vp3 residues might not be readily exposed at the surface of the fusion protein. It was found that Vp3 also binds to single-stranded DNA and RNA with roughly 4-fold greater affinity than to duplex DNA, a result similar to that reported for SV40 Vp1 (10). The nucleic acid binding $K_d$ values of GST-Vp3 were lower than that reported for the interaction of bacterially expressed polyoma
Vp1 with DNA (10⁻¹¹ m: 11). This is somewhat surprising since the interaction between GST-Vp3 and DNA was more stable in salt than that reported between Vp1 and DNA (11). Under alkaline conditions, SV40 Vp1 and Vp2 dissociate from the viral DNA, while most of the Vp3 and histones remain complexed with the minichromosome (13), suggesting that Vp3 might interact even more strongly than Vp1 with DNA.

However, the evidence for alkaline dissociation could reflect the effect on the overall chemical bonds between the three viral proteins, and not necessarily between the viral proteins and DNA.

Several stretches within the last 35 amino acids of Vp3 share significant sequence similarity with histones H1, H2A, and H2B (17). The carboxy-terminal 13 residues of Vp3, shown in this report to be critical for DNA binding, are 64% identical in amino acid sequence over an 11-residue stretch to a region near the amino terminus of histone H2A which is thought to make contact with the linker DNA connecting adjacent nucleosomes, thereby aiding in condensation (35, 36). Thus, this region of Vp3 might not only bind to DNA, but might also condense it in a manner similar to the H2A region in histones.

The ability of the F6 to compact DNA, as viewed by the increased mobility of long DNA fragments in the gel shift is intriguing. That the GST-Vp3 fusion protein caused only gel retardation of both short and long DNA fragments in the gel shift further suggests that the existence of the last 40 residues of Vp3 as a tetramer in F6 enables F6 to compact DNA. Judging from the result that DNA fragments shorter than 1.0 kb do not yield the fast migrating species when they interact with F6, fragment size appears to matter for compaction. We do not know how three distinct species are generated with long DNA, but a large fusion protein containing four histone-like domains, five from a Vp1 pentamer and one from either Vp2 or Vp3, are expected to be involved in the compaction of an SV40 minichromosome. Whether the structural proteins bind to the minichromosome as a complex or sequentially is unknown. An in vitro experiment in which the conversion of polyoma Vp1 pentamers into virion-like particles, in the absence of Vp3, is blocked in the presence of DNA suggests that Vp3 may mediate the encapsidation of the minichromosome by Vp1 (11). Thus, we propose that the SV40 minichromosome interacts first with Vp2 and Vp3 molecules through a Vp2/Vp3-DNA binding signal to nucleate the minichromosome compaction. Interaction of this complex with five Vp1-DNA binding domains within a pentamer could position the pentamer relative to the minichromosome allowing the propagation of the assembly process. Our interpretation that virion formation occurs in this distinct order is in agreement with proposed models involving progressive virion assembly (16, 38-43).

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