JOURNAL OF VIROLOGY, Dec. 1991, p. 6998–7003
0022-538X/91/126998-06$02.00/0

Vol. 65, No. 12

# Determination of the Origin-Specific DNA-Binding Domain of Polyomavirus Large T Antigen

NOELLE-ANNE SUNSTROM,[1] NICHOLAS H. ACHESON,[1]* AND JOHN A. HASSELL[2]

*Department of Microbiology and Immunology, McGill University, Montreal, Quebec H3A 2B4,[1] and Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario L8S 4K1,[2] Canada*

Received 4 April 1991/Accepted 9 September 1991

**To map the DNA-binding domain of polyomavirus large T antigen, we constructed a set of plasmids coding for unidirectional carboxy- or amino-terminal deletion mutations in the large T antigen. Analysis of origin-specific DNA binding by mutant proteins expressed in Cos-1 cells revealed that the C-terminal boundary of the DNA-binding domain is at or near Glu-398. Fusion proteins of large T antigen lacking the first 200 N-terminal amino acids bound specifically to polyomavirus origin DNA; however, deletions beyond this site resulted in unstable proteins which could not be tested for DNA binding. Testing of point mutants and internal deletions by others suggested that the N-terminal boundary of the DNA-binding domain lies between amino acids 282 and 286. Taken together, these results locate the DNA-binding domain of polyomavirus large T antigen to the 116-amino-acid region between residues 282 and 398.**

Polyomavirus large T antigen is required for the initiation of viral DNA replication and the regulation of early mRNA synthesis (5, 15). These functions of large T antigen are thought to require direct binding of the protein to sequences within the noncoding regulatory region of the viral genome (7, 10, 13). Polyomavirus large T antigen binds to three high-affinity binding sites, designated A, B, and C, which are intermingled with elements that control early transcription, and to three weaker binding sites, designated 1, 2, and 3, located within the core origin for DNA replication. Each binding site is characterized by the presence of two to four copies of the pentanucleotide sequence 5′-G(A/G)GGC-3′ (7, 8, 30).

We are particularly interested in defining the functional domains of polyomavirus large T antigen important for DNA replication and transcriptional control and in comparing these to domains found in other papovavirus large T antigens. Despite a wealth of information regarding the many domains of the large T antigen of simian virus 40 (SV40) (12), those of other members of the papovavirus family are poorly characterized. This information could lend insight into the evolution of papovaviruses and might help in understanding the species specificity of the DNA replication activity of large T antigen (3).

Here we report the construction and characterization of amino-terminal and carboxy-terminal deletion mutants of polyomavirus large T antigen. To define the specific DNA-binding domain of this protein, we expressed truncated large-T-antigen proteins transiently in Cos-1 cells (17) and measured specific DNA-binding activity in nuclear extracts by an immunoprecipitation assay (24, 25).

**Construction of deletion mutants of large T antigen.** We used the expression vector pPyLT (24) (Fig. 1A), in which a cDNA fragment coding for large T antigen (nucleotides [nt] 153 to 2962) was cloned as an *Xho*I-*Bam*HI fragment. Synthesis of large-T-antigen mRNA is driven by the adenovirus major late promoter, and transcripts initiated from this promoter include the adenovirus major late tripartite leader sequence. Immediately downstream of the large-T-antigen coding region are the polyadenylation signals of both polyomavirus and SV40. The vector also contains the SV40 origin of DNA replication to allow template amplification after transfection of Cos-1 cells.

A schematic representation of the steps involved in generating large-T-antigen peptides truncated at the C terminus is shown in Fig. 1B. The plasmid pPyLT was linearized by digestion with *Bam*HI. The 3′ recessed ends were filled in with alpha-phosphorothioate deoxynucleosides at a final concentration of 0.04 mM by using Klenow DNA polymerase. This step prevents subsequent digestion of the blocked end of the plasmid by exonuclease III (19). The plasmid was then digested with *Xba*I to generate a DNA fragment containing the large-T-antigen coding region up to the *Xba*I site at nt 2479 (amino acid 641 of large T antigen); nucleotide numbers are assigned as for the A2 strain of polyomavirus (34). This DNA (0.2 μg/μl) was then digested with exonuclease III (10 U/μl) at 37°C. Aliquots were removed at 30-s intervals to tubes containing S1 nuclease (0.2 U/μl), 0.4 M potassium acetate (pH 4.6), 0.3 M NaCl, and 1.4 mM zinc sulfate and incubated at 25°C for 30 min. After inactivation of the S1 nuclease (70°C for 10 min), the Klenow fragment of DNA polymerase and all four deoxynucleoside triphosphates were added to flush DNA ends. A 48-mer oligonucleotide (stop linker) containing translational stop codons in all three reading frames (Fig. 1C), flanking a sequence encoding the lac repressor binding site (2), was ligated to the truncated cDNA. This was to ensure production of proteins containing two or fewer foreign amino acids beyond the deletion end point at their C termini. The ligation mixtures were used to transform competent *Escherichia coli* DH5, which was plated on minimal agar medium containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (0.04 mg/ml) and IPTG (isopropyl-β-D-thiogalactopyranoside) (2 mg/ml). Development of blue color in colonies indicates the presence of the lac repressor binding site and signals the presence of the stop codon oligonucleotide in the recombinant plasmid. A number of subclones from each time point was examined by digestion with restriction endonucleases to determine the end point of the deletions. The dideoxy method of sequencing (31) was used to determine the extent of deletions.

The generation of N-terminal deletions of large T antigen

* Corresponding author.

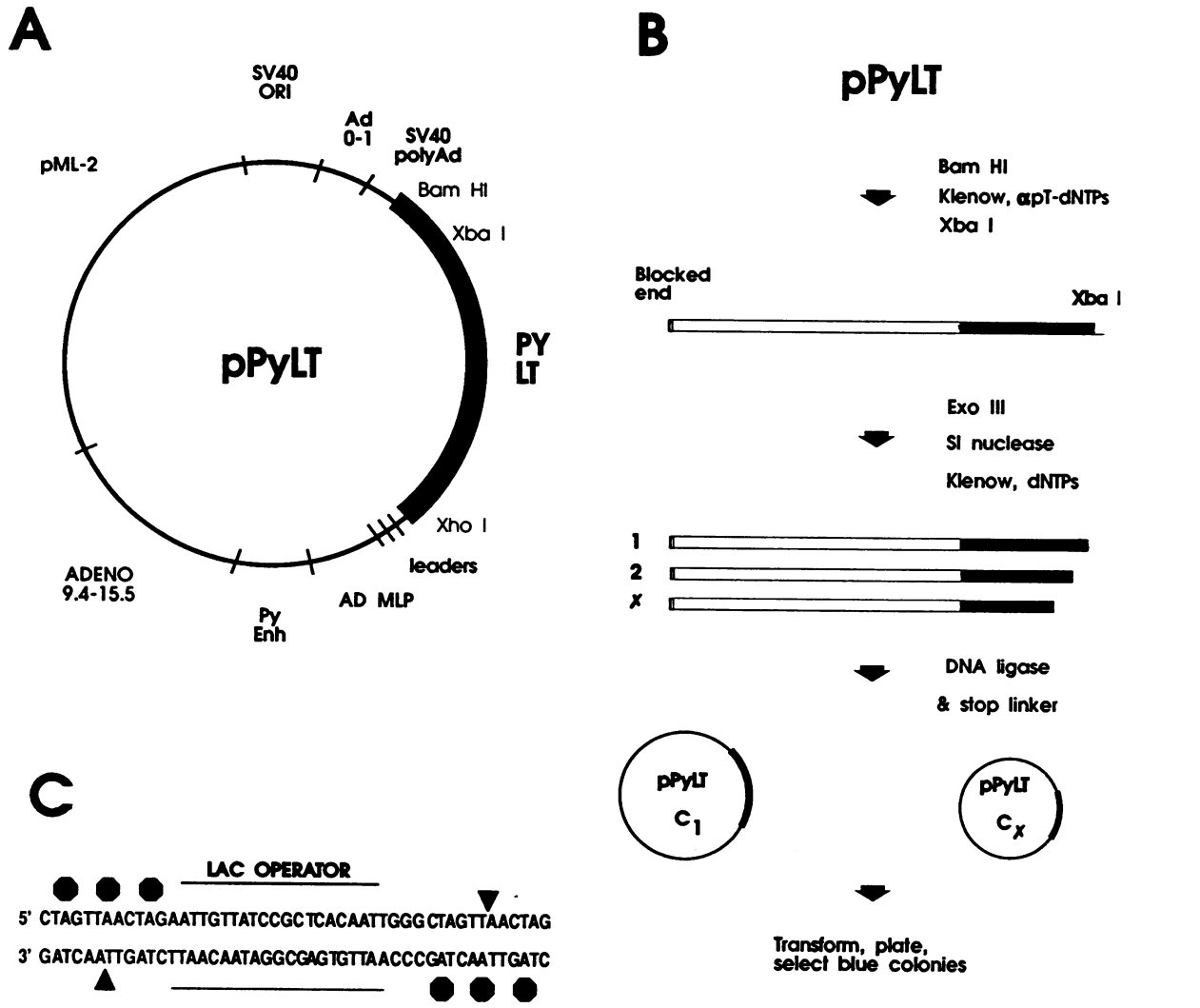


FIG. 1. Construction of carboxy-terminal deletion mutants of polyomavirus large T antigen. (A) Parent plasmid pPyLT includes large-T-antigen cDNA as an *Xho*I-to-*Bam*HI fragment (nt 154 to 2962) whose transcription is directed by the adenovirus major late promoter (AD MLP; nt 5986 to 6038) and tripartite leader (first, second and two-thirds of the third leader). SV40 polyAd, SV40 polyadenylation signal (nt 2770 to 2533); Ad 0–1, adenovirus left-hand end (0 to 1 map units, nt 1 to 357); SV40 ORI, SV40 origin of replication and enhancer region (nt 270 to 0 and 5243 to 5171); pML-2, plasmid pML-2 sequences (nt 650 to 4362); ADENO 9.4–15.5, adenovirus sequences from 9.4 to 15.5 map units (nt 3328 to 5788); Py Enh, polyomavirus enhancer region (nt 5039 to 5265). (B) Schematic representation of steps involved in the construction of carboxy-terminal deletion mutants of large T antigen. $C_1$, $C_x$: plasmids encoding fragments of large T antigen deleted at the C terminus and decreasing in size. (C) Oligonucleotide employed in the cloning procedure to facilitate screening of recombinant plasmids containing the lac repressor binding site (lac operator). Octagons represent stop codons in all three reading frames flanking the lac operator. Triangles indicate the position of unique *Hpa*I restriction sites.

required reintroduction of an in-frame AUG translational initiation codon upstream of the truncated coding region. This was achieved by ligating a synthetic DNA fragment (start linker) containing an ATG in the optimal context (22) to exonuclease III-digested blunt-ended DNAs. We also introduced in the start linker a sequence coding for an antigenic determinant of the influenza virus hemagglutinin (HA) protein (14), immediately downstream of the translational initiation codon. This allowed us to immunoprecipitate the resulting HA–large-T-antigen-fragment fusion proteins with an anti-HA antibody. The strategy used for the construction of amino-terminal deletion mutants of large T antigen was similar to that described for carboxy-terminal deletion mutants. The plasmid pPyLT was digested with *Xho*I to linearize the DNA at the N terminus of the large-T-antigen coding region. Exonuclease III digestion, S1 nuclease treatment, and blunt ending were carried out as described above. The DNA was then cleaved with *Bam*HI to release fragments containing the large-T-antigen coding region with deletions at its N-terminal end. These fragments were isolated from an agarose gel and ligated to the vector pPyLT, which had been previously cleaved with *Xho*I and *Bam*HI. Ligation was carried out in the presence of a chemically synthesized nonphosphorylated double-stranded start linker (one strand: 5′-TCGAGATGTACCCATACGA TGTTCCAGATTACGCTAGCTTGGGTGGTCCT-3′; complementary strand: 3′-CTACATGGGTATGCTACAAGGT CTAATGCGATCGAACCCACCAGGA-5′), which has at one end an *Xho*I recognition site (underlined) followed by the start codon for translation (ATG), followed by a DNA

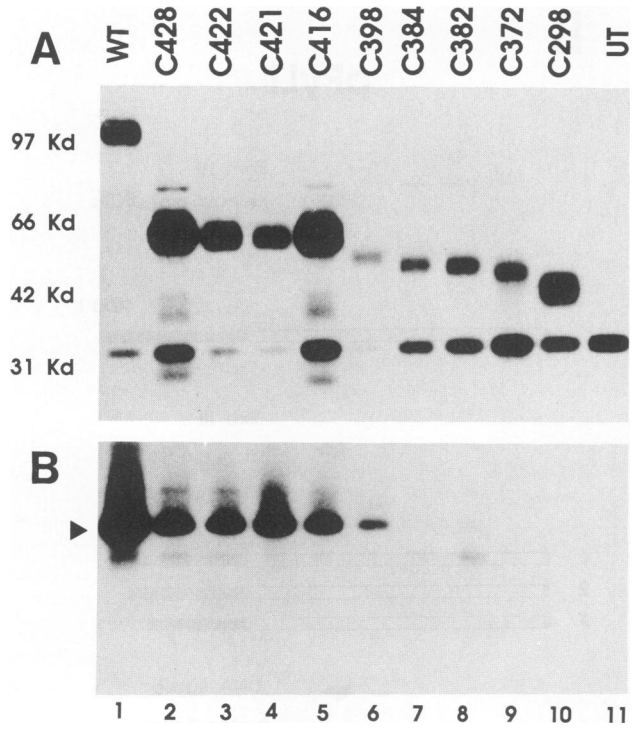


FIG. 2. (A) Immunoprecipitation by polyclonal antiserum of [$^{35}$S]methionine-labeled large-T-antigen fragments made in Cos-1 cells transfected with C-terminal deletion mutants. Proteins were analyzed by 10% polyacrylamide gel electrophoresis. WT, wild-type large T antigen; C428 to C298, C-terminal deletion mutants of large T antigen whose termini are at the numbered positions; UT, untransfected Cos-1 cells. The numbers on the left indicate molecular size in kilodaltons (Kd) determined from migration of marker proteins. (B) Specific DNA binding by large-T-antigen fragments described for panel A. Input DNA is *Hin*fI-digested recombinant polyomavirus DNA (pPHI-8). Arrow indicates the location of the 604-bp DNA fragment containing all large-T-antigen binding sites (the input DNA digestion mixture is not shown; see Fig. 3B).

sequence encoding a 14-amino-acid peptide of the influenza virus HA epitope (14), and at the other end is blunt ended. This three-part ligation was done at a molar ratio of vector: fragment:linker of 1:5:50. The ligation mix was used to transform competent *E. coli* DH5. Recombinant plasmids were screened for the size of deletions by digestion with *Bam*HI and *Xho*I. Those of interest were sequenced and plasmids which generate in-frame HA–large T-antigen fusion peptides were chosen.

**Expression of large T antigens containing C-terminal deletions.** Plasmid DNAs encoding large T antigen harboring deletions at the C terminus were analyzed for their ability to express large-T-antigen fragments after transfection into Cos-1 cells as described previously (3) by labeling total cellular proteins with [$^{35}$S]methionine as previously described (24). Proteins in Cos-1 cell extracts were immunoprecipitated with either the monoclonal antibody LT1 (Oncogene Science) or a polyclonal antiserum (obtained from ascites fluid of brown Norwegian rats bearing tumors induced by polyomavirus-transformed rat cells). Typical results are shown in Fig. 2A. In addition to large-T-antigen fragments, a cellular protein of approximately 35 kDa was regularly precipitated by the polyclonal antiserum. Different amounts of both large-T-antigen fragments and the cellular protein were found in different extracts; these variations may be due to different efficiency of labeling or extraction of proteins. We have not attempted to quantitate levels of large-T-antigen fragments made by each C-terminal mutant. However, all the C-terminal mutants expressed a unique species of large T antigen corresponding in size to that expected from the sequenced end points of the deletions. SV40 large T antigen, which is also present in Cos-1 cells, was not recognized by antibodies directed against polyomavirus large T antigen (Fig. 2A, untransfected cell extract).

**Specific DNA binding by large-T-antigen fragments containing C-terminal deletions.** The sequence-specific DNA-binding activity of the carboxy-terminal truncated large T antigens was examined by a modified McKay immunoprecipitation assay (24, 25). Nuclear lysates of transfected Cos-1 cells were mixed with a $^{32}$P-end-labeled *Hin*fI digest of the plasmid pPHI-8 (29). This *Hin*fI digest contains 20 fragments, one of which, 604 bp in length, contains the region of polyomavirus DNA (nt 5073 to 5296 and 1 to 385) to which large T antigen binds. Large T-antigen–DNA complexes were immunoprecipitated, and bound DNA was released from the large-T-antigen–antibody complex and fractionated on an agarose gel (Fig. 2B). Specific binding by large T antigen selectively precipitates the 604-bp fragment from the mixture. Large-T-antigen fragments with C termini up to amino acid 398 were capable of specific binding to target DNA (Fig. 2B, lanes 2 to 6). However, the deletion of 14 additional amino acids from the carboxy terminus of large T antigen (C384) resulted in a protein which was unable to bind to the origin fragment (lane 7). These results locate the carboxy terminus of the DNA-binding domain between amino acids 384 and 398. This domain lies upstream of a putative metal-binding region (zinc finger) in polyomavirus large T antigen (amino acids 452 to 472) (21). Although a point mutation within the zinc finger was shown to abolish DNA-binding activity (4), our results demonstrate that it does not contribute directly to the specific DNA-binding activity of large T antigen.

It has been shown that SV40 and polyomavirus large T antigen recognize and bind to similar sequence motifs in DNA (29, 32). Therefore, it was important to ensure that all DNA-binding studies were conducted in DNA excess, so that SV40 large T antigen present in Cos-1 cell extracts would not compete with polyomavirus large T antigen for DNA binding. We observed that the unbound fraction of DNA remaining in the supernatant after precipitation of the immune complexes contained greater than 90% of the origin-containing fragment (not shown). Also, DNA-binding assays using antibodies directed against SV40 large T antigen resulted in only a small portion of the input polyomavirus origin-containing DNA fragment being bound (not shown).

**Expression of large T antigens containing N-terminal deletions.** HA–large-T-antigen fusion proteins lacking up to the first 175 amino acids of large T antigen (N175) were recognized by the monoclonal antibody LT1 and a rat polyclonal antiserum, both specific to polyomavirus large T antigen, as well as by the anti-HA monoclonal antibody 12CA5 (Fig. 3A, lanes 4 to 9). Mutants N188 and N189 produced proteins which were immunoprecipitated by the polyclonal antiserum and anti-HA but not by the monoclonal antibody LT1 (lanes 10 to 15). These mutants therefore lack the antigenic determinant recognized by the monoclonal antibody LT1 (11).

Larger deletions at the N terminus of large T antigen resulted in the progressive inability to detect these proteins by immunoprecipitation of extracts from [$^{35}$S]methionine-labeled Cos-1 cells. Although a small amount of N201 was

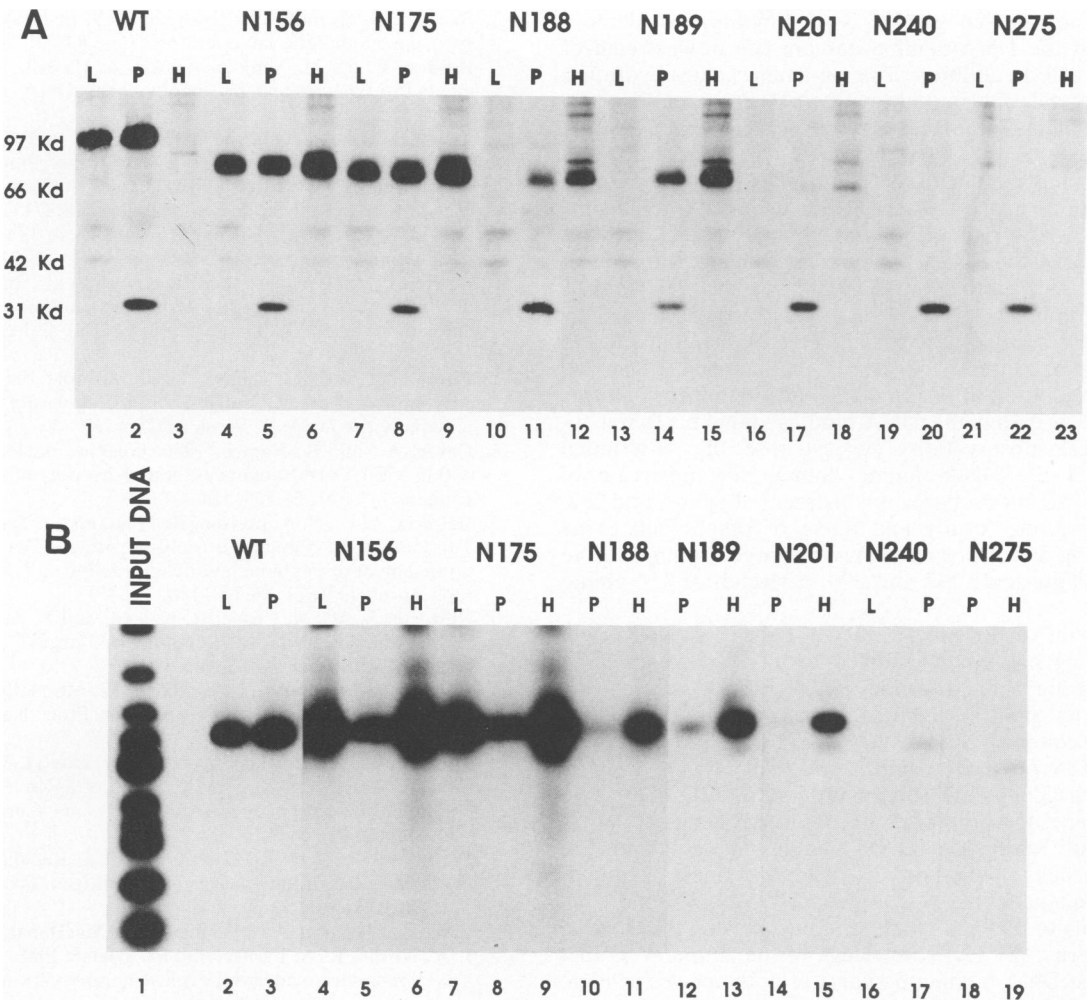


FIG. 3. (A) Immunoprecipitation of [$^{35}$S]methionine-labeled large-T-antigen fragments made in Cos-1 cells transfected with N-terminal deletion mutants. Proteins were analyzed as described in the legend to Fig. 2. WT, wild-type large T antigen; N156 to N275: N-terminal deletion mutants of large T antigen whose N-terminal amino acids are at the numbered positions. Antibodies used: L, LT1 monoclonal antibody; P, polyclonal antiserum 3e1; H, 12CA5 monoclonal antibody directed against influenza virus HA epitope. (B) Specific DNA binding by N-terminal mutants of large T antigen. Input DNA is *Hin*fI-digested recombinant polyomavirus DNA (pPHI-8). Labels above each lane designate the antibody used to precipitate the large-T-antigen–DNA complex.

immunoprecipitated by anti-HA antibody 12CA5 and by the polyclonal antiserum (Fig. 3A, lanes 17 and 18), no specific protein band was visible after immunoprecipitation of N240 (this construct does not contain the HA epitope) or N275 (lanes 19 to 23). We also obtained other in-frame N-terminal deletion mutants of large T antigen which map downstream of N201 (N221, N278, and N303), but none of these mutant proteins was detectable by immunoprecipitation (data not shown).

It remained possible that deletions beyond amino acid 201 masked the N-terminal HA epitope by burying it within an inaccessible region of the protein. Therefore, we attempted immunoprecipitation with a polyclonal antiserum directed against a peptide containing amino acids 273 to 725 of large T antigen (30a). Whereas full-length large-T-antigen and N-terminal mutants N156, N175, N188, N189, and N201 were detected after immunoprecipitation using this antiserum, large-T-antigen mutants with larger deletions at the N terminus were not detected (data not shown). We concluded from these observations that large-T-antigen fragments lacking more than approximately 200 amino acids at their amino termini are unstable in Cos-1 cells.

**Specific DNA binding by large-T-antigen fragments with N-terminal deletions.** Large-T-antigen mutants N156 and N175 were capable of specific origin DNA binding comparable to that of full-length large T antigen, independent of the antibody used to precipitate the protein-DNA complex (Fig. 3B, lanes 2 to 9). In addition, mutants N188, N189, and N201, which produced somewhat smaller amounts of protein, were nonetheless capable of specific origin binding (lanes 10 to 15). Therefore, amino acids 1 to 200 of large T antigen are dispensable for specific DNA binding. As mentioned previously, proteins with N-terminal deletions beyond amino acid 201 could not be detected in transfected Cos-1 cells and therefore the N-terminal boundary of the specific DNA-binding domain could not be defined more precisely by using this system.

Several mutations in polyomavirus large T antigen have been tested previously for their effects on specific DNA-binding activity (6, 26, 36). The deletion of amino acids 130

to 260 did not abolish specific DNA binding (26), demonstrating that the DNA-binding domain lies downstream of amino acid 260. In addition, a single-amino-acid substitution at position 282 did not alter DNA-binding activity (6). However, single-amino-acid changes at positions 293, 297, and 300 led to loss of DNA-binding activity, as did deletion of 11 amino acids at positions 300 to 310 (6). Introduction of point mutations in the G(A/G)GGC consensus binding sites at the replication origin of polyomavirus DNA led to loss of the capacity of these DNAs to be replicated. Mutation of Asp-286 to Asn partially restored the capacity of large T antigen to replicate DNAs with mutated origins (36). This implies that binding of large T antigen to the mutated origin (which was not directly measured in that study) was improved by mutation at position 286 and, therefore, that this amino acid is within the DNA-binding domain. Taken together, these observations suggest that the N-terminal boundary of the DNA-binding domain lies upstream of amino acid 286 but probably downstream of amino acid 282. In summary, our results and those of others define the DNA-binding domain of polyomavirus large T antigen to lie between amino acids 282 and 398, a stretch of 116 amino acids.

The domain responsible for SV40 large-T-antigen origin DNA binding has been localized to a 114-residue region between amino acids 132 and 246 (1, 28, 33, 35). When polyomavirus and SV40 large T antigens are aligned to maximize sequence similarity, Val-132 of SV40 is aligned with Asp-286 of polyomavirus and Glu-246 of SV40 is aligned with Glu-398 of polyomavirus (16, 18). These two regions share 45% direct amino acid sequence identity. Conservation within the DNA-binding domain would be expected because both proteins recognize similar pentanucleotide sequences in DNA targets (8, 9, 20, 30) and each protein binds to the high-affinity binding sites on the DNA of the other virus (29, 32). Knowledge of the amino acids that make up the DNA-binding domains of both species of large T antigen should aid efforts to map more precisely those amino acids whose side chains contact DNA directly (33).

The mechanism by which large T antigen recognizes and binds to its target sites on DNA is not known. Little or no similarity exists between the DNA-binding domain of polyomavirus or SV40 large T antigen and that of other DNA-binding proteins (33). Presumably, large-T-antigen monomers recognize each pentanucleotide, and the strength of binding is influenced by protein-protein interactions between T-antigen molecules (8, 9, 27). ATP and other nucleotides increase the affinity of polyomavirus large T antigen for its target DNA (23); ATP may affect binding by altering these protein-protein interactions. If such cooperative interactions do take place, it will be of interest to determine the region of polyomavirus large T antigen involved by analyzing mutants such as those described here.

We thank Lynn Kelly for excellent technical assistance and Carol Prives for a gift of antiserum.

This research was supported by the National Cancer Institute of Canada (to J.A.H.) and the Medical Research Council of Canada (grant no. MT-7281 to N.H.A.). N.-A.S. was supported by a graduate fellowship from the Cancer Research Society, Inc. (Montreal).

## REFERENCES

1. **Arthur, A. K., A. Höss, and E. Fanning.** 1988. Expression of simian virus 40 T antigen in *Escherichia coli*: localization of T-antigen origin DNA-binding domain to within 129 amino acids. J. Virol. **62**:1999–2006.
2. **Bautista, D. S., and F. L. Graham.** 1989. Insertional mutagenesis using a synthetic lac operator. Gene **82**:201–208.
3. **Bennett, E. R., M. Naujokas, and J. A. Hassell.** 1989. Requirements for species-specific papovavirus DNA replication. J. Virol. **63**:5371–5385.
4. **Bergqvist, A., M. Nilsson, K. Bondeson, and G. Magnusson.** 1990. Loss of DNA-binding and new transcriptional transactivation function in polyomavirus large T-antigen with mutation of zinc finger motif. Nucleic Acids Res. **18**:2715–2720.
5. **Cogen, B.** 1978. Virus-specific early RNA in 3T6 cells infected by a tsa mutant of polyoma virus. Virology **85**:220–230.
6. **Cowie, A., J. de Villiers, and R. Kamen.** 1986. Immortalization of rat embryo fibroblasts by mutant polyomavirus large T antigens deficient in DNA binding. Mol. Cell. Biol. **6**:4344–4352.
7. **Cowie, A., and R. Kamen.** 1984. Multiple binding sites for polyomavirus large T antigen within regulatory sequences of polyomavirus DNA. J. Virol. **52**:750–760.
8. **Cowie, A., and R. Kamen.** 1986. Guanine nucleotide contacts within viral DNA sequences bound by polyomavirus large T antigen. J. Virol. **57**:505–514.
9. **DeLucia, A. L., B. A. Lewton, R. Tjian, and P. Tegtmeyer.** 1983. Topography of simian virus 40 A protein-DNA complexes: arrangement of pentanucleotide interaction sites at the origin of replication. J. Virol. **46**:143–150.
10. **Dilworth, S. M., A. Cowie, R. Kamen, and B. E. Griffin.** 1984. DNA binding activity of polyomavirus large T antigen. Proc. Natl. Acad. Sci. USA **81**:1941–1945.
11. **Dilworth, S. M., and B. E. Griffin.** 1982. Monoclonal antibodies against polyoma virus tumor antigens. Proc. Natl. Acad. Sci. USA **79**:1059–1063.
12. **Fanning, E., J. Schneider, A. Arthur, A. Höss, I. Moarefi, and S. Modrow.** 1989. Structure and function of SV40 large T antigen; communication between functional domains. Curr. Top. Microbiol. Immunol. **144**:9–19.
13. **Farmerie, W. G., and W. R. Folk.** 1984. Regulation of polyomavirus transcription by large tumor antigen. Proc. Natl. Acad. Sci. USA **81**:6919–6922.
14. **Field, J., J.-I. Nikawa, D. Broek, B. MacDonald, L. Rodgers, I. A. Wilson, R. A. Lerner, and M. Wigler.** 1988. Purification of a *RAS*-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. Mol. Cell. Biol. **8**:2159–2165.
15. **Franke, B., and W. Eckhart.** 1973. Polyoma gene function required for viral DNA synthesis. Virology **55**:127–135.
16. **Friedmann, T., A. Esty, P. LaPorte, and P. Deininger.** 1979. The nucleotide sequence and genome organization of the polyoma early region: extensive nucleotide and amino acid homology with SV40. Cell **17**:715–724.
17. **Gluzman, Y.** 1981. SV40-transformed simian cells support the replication of early SV40 mutants. Cell **23**:175–182.
18. **Griffin, B. E.** 1982. Structure and genomic organization of SV40 and polyomavirus, p. 61–123. *In* J. Tooze (ed.), DNA tumor viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–355.
20. **Jones, K. A., and R. Tjian.** 1984. Essential contact residues within SV40 large T antigen binding sites I and II identified by alkylation-interference. Cell **36**:155–162.
21. **Klug, A., and D. Rhodes.** 1987. "Zinc fingers": a novel protein motif for nucleic acid recognition. Trends Biochem. Sci. **12**:464–469.
22. **Kozak, M.** 1984. Point mutations close to the AUG initiator codon affect the efficiency of translation of rat preproinsulin in vivo. Nature (London) **308**:241–246.
23. **Lorimer, H. E., E. H. Wang, and C. Prives.** 1991. The DNA-binding properties of polyomavirus large T antigen are altered by ATP and other nucleotides. J. Virol. **65**:687–699.
24. **Massie, B., Y. Gluzman, and J. A. Hassell.** 1986. Construction of a helper-free recombinant adenovirus that expresses polyomavirus large T antigen. Mol. Cell. Biol. **6**:2872–2883.

25. **McKay, R. D. G.** 1981. Binding of a simian virus 40 large T antigen related protein to DNA. J. Mol. Biol. **145**:471–488.
26. **Nilsson, S. V., and G. Magnusson.** 1984. Activities of polyomavirus large-T-antigen proteins expressed by mutant genes. J. Virol. **51**:768–775.
27. **Parsons, R. E., J. E. Stenger, S. Ray, R. Welker, M. E. Anderson, and P. Tegtmeyer.** 1991. Cooperative assembly of simian virus 40 T-antigen hexamers on functional halves of the replication origin. J. Virol. **65**:2798–2806.
28. **Paucha, E., D. Kalderon, R. W. Harvey, and A. E. Smith.** 1986. Simian virus 40 origin DNA-binding domain on large T antigen. J. Virol. **57**:50–64.
29. **Pomerantz, B. J., and J. A. Hassell.** 1984. Polyomavirus and simian virus 40 large T antigens bind to common DNA sequences. J. Virol. **49**:925–937.
30. **Pomerantz, B. J., C. R. Mueller, and J. A. Hassell.** 1983. Polyomavirus large T antigen binds independently to multiple, unique regions on the viral genome. J. Virol. **47**:600–610.
30a. **Prives, C.** Personal communication.
31. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
32. **Scheller, A., and C. Prives.** 1985. Simian virus 40 and polyomavirus large tumor antigens have different requirements for high-affinity sequence-specific DNA binding. J. Virol. **54**:532–545.
33. **Simmons, D. T., W.-K. Kyungok, and W. Young.** 1990. Identification of simian virus 40 T-antigen residues important for specific and nonspecific binding to DNA and for helicase activity. J. Virol. **64**:4858–4865.
34. **Soeda, E., J. R. Arrand, N. Smolar, J. E. Walsh, and B. E. Griffin.** 1980. Coding potential and regulatory signals of the polyoma virus genome. Nature (London) **283**:445–453.
35. **Strauss, M., P. Argani, I. J. Mohr, and Y. Gluzman.** 1987. Studies on the origin-specific DNA-binding domain of simian virus 40 large T antigen. J. Virol. **61**:3326–3330.
36. **Tang, W.-J., and W. R. Folk.** 1989. Asp-286→Asn-286 in polyomavirus large T antigen relaxes the specificity of binding to the polyomavirus origin. J. Virol. **63**:242–249.