The high affinity binding site on polyoma virus DNA for the viral large-T protein

Patrick Gaudray*, Chiara Tyndall+, Robert Kamen+ and François Cuzin*

*Centre de Biochimie du CNRS, Université de Nice, 06034 Nice, France, and +Transcription Laboratory, Imperial Cancer Research Fund Laboratories, P.O. Box 123, Lincoln's Inn Fields, London, UK

Received 27 August 1981

ABSTRACT.

In order to map the high affinity binding site for the viral large-T protein on polyoma virus DNA, we have developped an assay which does not require purified protein. It is based on the specific elution of the large-T ATPase activity from calf thymus DNA cellulose by recombinant DNA molecules including known sequences of the viral DNA. Using this assay, a high affinity binding site has been mapped on the early region side of the ori region. Binding requires the integrity of a sequence /AGAGGC/TTCC/AGAGGC/ (nucleotides 49 to 64 in the DNA sequence of the A2 strain). Similar repeats of a PuGPuGGC sequence within less than 20 bases are not found within the viral coding regions, but are strikingly common in the control regions of papovaviruses and other eukaryotic DNAs.

INTRODUCTION.

The polyoma virus large-T protein (1) acts as an initiator of viral DNA synthesis (2,3) and as a regulator of early mRNA synthesis (4). The protein exhibits an ATP-phosphohydrolase (ATPase) activity and the ability to bind double-stranded DNA (5).

Specific recognition of defined deoxynucleotide sequences by regulatory proteins was previously demonstrated in a number of prokaryotic systems (see ref. 6 and 7 for reviews). In the case of the eukaryotic SV40 virus, it was shown by R. Tjian (8,9) that both the adenovirus-SV40 D2 hybrid protein and the authentic viral large-T antigen bind sequentially to three sites in the region of the viral DNA where the replication origin (ori) had been previously localized.

The replication origin of polyoma DNA was located at 71 map units by electron microscopy and pulse labeling experiments (10-12). The HpaII 3/5 restriction site chosen as the beginning of the numbering system for the polyoma virus sequence that we use throughout this paper (13) is within the replication origin region (see Figure 4). A sequence of close homology with the SV40 ori sequence occurs from nucleotide 5264 to 14 and includes a large inverted repeat (13,14).

Studies on the binding of SV40 large-T protein to its DNA were greatly facilitated by the availability of SV40-adenovirus hybrids which produced large amounts of the protein (8,9). It was thus possible to obtain sufficient quantities of highly purified material suitable for the study of its binding to the viral DNA. There is no analogous source of the polyoma virus large-T protein, and, although it has been extensively purified from infected cells (5), only small amounts of protein could be obtained. We have therefore devised a competition method in which the DNA-binding proteins from crude extracts of polyoma virus-infected 3T6 cells are first adsorbed to double-stranded calf thymus DNA cellulose. Proteins with higher affinity for defined sequences in the viral DNA can then be specifically eluted at relatively low ionic strength in the presence of subgenomic viral DNA fragments, cloned and amplified in bacterial plasmids. Partition of the large-T protein between the cellulose-bound non specific sequences and the viral fragments in solution was monitored by assaying its ATP hydrolytic activity. This enzymatic activity was demonstrated previously to be associated with the large-T gene product on the basis of a series of stringent criteria: it co-purifies with the large-T antigen, it is specifically inhibited by anti-polyoma T antigen antibodies and it exhibits an increased thermolability in vitro in the case of tsa mutants (5).

MATERIAL AND METHODS.

Cells and viruses, purification of polyoma large-T protein: the cell lines and culture conditions, the virus strains and the procedure for purification of polyoma large-T protein were described previously (5).

ATPase assay: ATPase activity was assayed by the enzyme-coupled assay described by Gache et al. (15).

Molecular cloning of polyoma DNA molecules: the pBR322-polyoma recombinants listed in Table 1 were used. Map positions of subgenomic fragments are shown in Figure 3. Plasmids pPY-1, pPY-2, pTS25E, pPC-2, pPC-10, pMC-2 and pMC-3 were constructed in our laboratory following the usual procedures (see ref 16 for review) (P. Clertant and M. Canning, unpublished results). Plasmids pKOH-3, RIDO-1 and pSVI-2 were kindly provided to us by Drs. K. O'Hare, R. Zorob and T. Soussi respectively.

Deletion mutant plasmids of the pdl2000 series (Table 1) were generated from full-length BamHI linear viral DNA cloned in the BamHI site of vector pAT153 by introduction of an XhoI linker into the PvuII site at nucleotide 5130 and subsequent Bal31 digestion from this new unique cleavage site. Each

Table 1: Bacterial vectors carrying polyoma DNA sequences.

PLASMID	INSERTED POLYOMA DNA SEQUENCES
(i) genomic viral DNA:	
pPY-1	Genomic wild type A2 DNA cleaved at BamHI site
pPY-2	Genomic wild type A3 DNA cleaved at BamHI site
pTS25E	Genomic tsa25E (A3) DNA (30) cleaved at BamHI site
pPY-45	Genomic dl45 (A3) DNA (deletion nucl. 1074-1140) (31) cleaved at BamHI site
(ii) subgenomic fragments from A2 strain DNA (see Figure 3):	
pPC-10	PstI fragment 1 (489-2360)
pPC-2	PstI fragment 2 (4226-488)
pMC-2	EcoRI - BamHI (1561-4632)
pMC-3	BamHI - EcoRI (4633-1560)
pKOH-3	HpaII fragment 3 (4410-5291)
RIDO-1	HaeII - HincII (99-2963)
(iii) deletion mutants (A2 strain DNA):	
(1) pdl2020P	Deletion of PvuII fragment 4 (5130-5264)
(2) Deletions extending from the late into the early region, ending:	
pdl2033	between nucleotides 5270 and 5279
pdl2088	" " 5290 " 1
pdl2019	at nucleotide 36 (*)
pdl2086	" " 53 (*)
pdl2015	" " 68 (*)
pdl2013	" " 98 (*)
pdl2018	" " 98 (*)
pdl2024	" " 100 (± 5)
pdl2045	" " 194 "

(*) end points of these deletions were determined from the DNA sequence.

mutant has an XhoI linker at the site of the deletion. Details of the construction and characterization of these mutants will be presented elsewhere (C. Tyndall and R. Kamen, manuscript in preparation).

Biohazards associated with the experiments performed in France have been examined previously by the French National Committee and the experiments were carried out according to the rules established by this Committee.

Large-T high affinity binding assay: ca 10^8 3T6 cells were infected with polyoma virus at a multiplicity of 10 PFU/cell. They were lyzed in 2 ml of B buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA, 1 mM dithiotreitol, 10% (vol/vol) glycerol) containing 0.3 M KCl and 0.5% (vol/vol) NP 40. After 15 min at 0°C with occasional stirring, the extract was diluted 10-fold with B buffer

containing 0.3 M KCl and clarified by centrifugation (15,000 x g, 15 min). It was then adsorbed batchwise on 1 ml of DNA-cellulose (packed volume), containing 1 mg of double-stranded calf thymus DNA, previously equilibrated in the same buffer for 2 h at 0°C. DNA cellulose was poured into a 2 ml column and washed overnight with 40 ml of B buffer supplemented with 0.3 M KCl. It was resuspended in 10 ml of the same buffer, divided in aliquots (0.1 ml packed volume) and decanted. Elution was performed by adding 0.1 ml of B buffer containing 0.3 M KCl and DNA for 1 h at 4°C. After centrifugation (11,000 x g, 2 min), the supernatant was filtered on glass fiber filters and assayed for ATPase activity.

DNA determination: concentrations were determined by ultraviolet spectrophotometry and ethidium bromide fluorescence after agarose gel electrophoresis.

RESULTS.

Elution of polyoma virus large-T ATPase from calf thymus DNA-cellulose in the presence of viral DNA.

In the experiment shown in Figure 1, a protein extract prepared from polyoma virus-infected 3T6 cells was first incubated with double stranded calf thymus DNA-cellulose. Protein binding to DNA-cellulose and subsequent washing were performed in the presence of 0.3 M KCl, below the molarity which elutes large-T (5). The DNA cellulose was then incubated with the same buffer containing plasmid pBR322 DNA, then with a solution of polyoma virus DNA. For 1 mg of cellulose-bound calf thymus DNA, the amounts of DNA in solution varied from 5 to 10 µg. A significant level of ATPase activity was observed

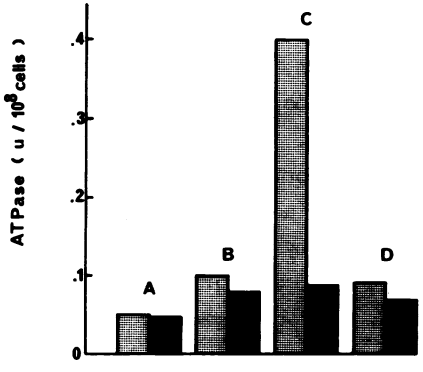

Figure 1. Elution of large-T ATPase from calf thymus DNA cellulose in the presence of viral DNA.

Calf thymus DNA-cellulose was loaded with DNA-binding proteins from polyoma virus-infected cells (see Methods). 0.1 ml of packed DNA cellulose was then incubated in succession for 1 hr at 4°C in a final volume of 0.2 ml of B buffer containing 0.3 M KCl (A), then in 0.2 ml of the same buffer containing pBR322 DNA (7.5×10^{-10} M) (B), polyoma virus DNA (5.5×10^{-10} M) (C) and 1 M KCl in the absence of DNA (D). ATPase activity was assayed in the presence of hamster immunoglobulin (▦ : non immune, ■ : anti-polyoma T antigen).

only in the fractions eluted in the presence of polyoma DNA. As in the case of the purified large-T protein, this activity was inhibited (ca 80%) by preincubation with anti-polyoma T antigen immunoglobulins. Specific elution of the viral enzyme in the presence of polyoma sequences was confirmed by the fact that no further release of large-T ATPase activity was detected upon subsequent application of high ionic strength buffer (1 M KCl), which would normally elute all of the large-T ATPase from the DNA cellulose (5).

Specificity and efficiency of large-T binding to polyoma DNA was monitored by comparing the dose/response curves for a recombinant plasmid DNA that includes polyoma sequences (pMC-3, see below) and a control DNA (pBR322). Results are shown in Figure 2.

Apparent $K_{0.5}$ values were 7×10^{-10} M and 5×10^{-8} M for pMC-3 and pBR322 DNA respectively. Assuming that there is only one site per DNA mole-

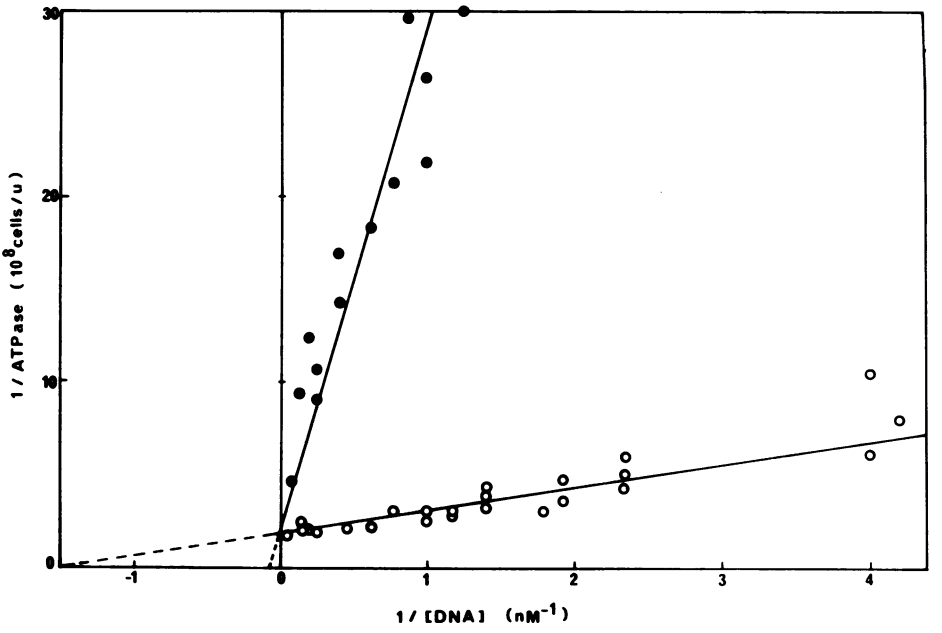

Figure 2. Elution of large-T ATPase from DNA-cellulose at various DNA concentrations.

DNA-cellulose was loaded as described in the Legend of Figure 1. 0.1 ml aliquots (packed volume) of DNA-cellulose were incubated as described in 0.2 ml of buffer containing various amounts of either pBR322 (●), or pMC-3 (○) plasmid DNA. The ATPase activity in the samples was plotted following the Lineweaver & Burk representation.

cule (see Discussion), and using the method of De Haseth et al. (17) for an estimation of the affinity of large-T for cellulose-bound DNA, the equilibrium dissociation constant of the large-T-viral DNA complex was estimated at about 2×10^{-11} M in 0.3 M KCl at pH 7.5. In all cases, Hill numbers were very close to 1, thus indicating that there is in fact one site - or one class of sites - per large-T molecule. All the following experiments were performed at a concentration of about 10^{-9} M DNA in solution, in order to reduce to less than 10% the level of non-specific binding (see Figure 2).

No significant ATPase activity was eluted under these conditions by DNA of plasmid pSVI-2 (Table 2), that carries the BstNI-G fragment of SV40 DNA.

Table 2: Binding of large-T ATPase to various plasmid DNAs.

DNA in solution	ATPase activity eluted (units/10^8 cells) (*)	Average
pBR 322	0.04	
pMC-2	0.03	
pPC-10	0.11	
RIDO-1	0.02	0.06 ± 0.04
pROH-3	0.07	
pPY-45	0.55	
pdl2020P	0.60	
pMC-3	0.30	0.50 ± 0.20
pPC-2	0.52	
pSVI-2	0.02	

(*) average of two to five experimental values for each DNA, corrected for background activity measured in the same buffer without DNA.

It includes the three high affinity binding sites of the SV40 large-T protein (8,9,18) (T. Soussi and A.M. de Recondo, personal communication). We can therefore conclude that, if polyoma large-T protein recognizes at all the SV40 ori region, it binds to these sequences with a relatively low affinity as compared with recognition of the ori region on the polyoma virus genome.

Large-T ATPase is efficiently eluted from calf thymus DNA only in the presence of subgenomic viral fragments that include the ori site.

The experiment reported in Figure 1 was repeated using recombinant plasmid DNAs including various subgenomic polyoma fragments (Table 1 and Figure 3). Results listed in Table 2 indicate a clear partition of polyoma sequences between two classes, one of them, but not the other, competing efficiently with the cellulose-bound DNA for the binding of large-T.

All efficient competitors include polyoma sequences in the vicinity of

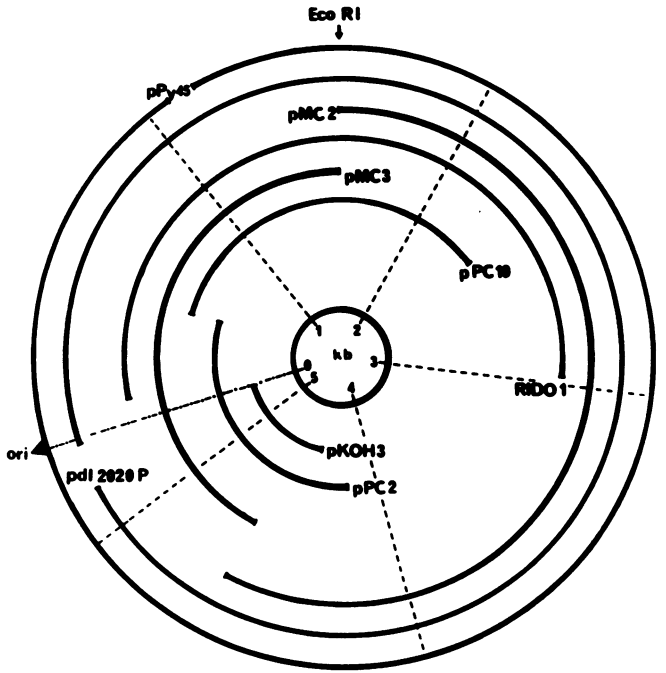

Figure 3. Subgenomic viral fragments inserted in recombinant plasmids.
Dotted lines : distances from the HpaII 3/5 junction expressed in kilobases (KB).

the previously recognized origin of replication (71 map units from the EcoRI site, close to the HpaII 3/5 junction at position 5292/1) (10-12). The viral sequence that is required for high affinity binding can be located from these data between nucleotides 1 (limit of the non-competitor HpaII-3 fragment (plasmid pKOH-3)) and 99 (limit of the viral sequence in plasmid RIDO-1) (Figures 3 and 4).

Localization of a primary recognition site between nucleotides 46 and 99.

Bendig, Thomas and Folk (19) concluded from studies on viable deletions starting from the HaeII (nucleotide 84) and BglI (nucleotide 87) sites toward the origin to the existence of a strict boundary at nucleotide 70 and suggested that "this boundary may demarcate a sequence which is of considerable importance to the virus". A remarkable sequence is in fact found just to the left of this boundary, within the high affinity region defined above. In polyoma A2 DNA (strand in the sense of early mRNA), a direct repeat of two AGAGGC hexanucleotides is found between nucleotides 49 and 64, separated by a group of four pyrimidines (TTCC). A third AGAGGC sequence is found immediately to the left at nucleotide 38. The nucleotide

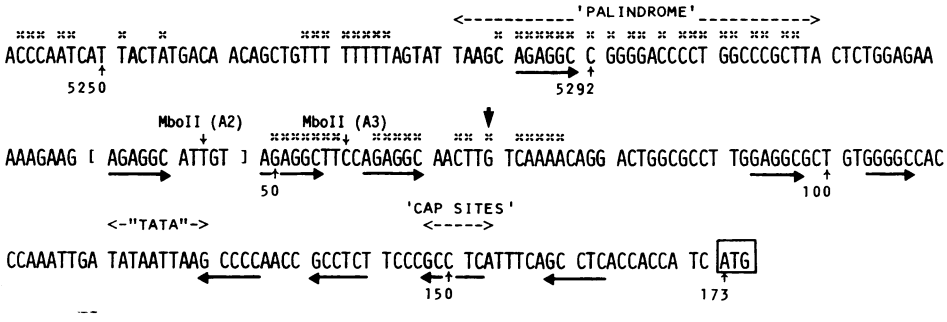

Figure 4. Nucleotide sequence around the origin of replication of polyoma virus DNA (14).

--- PALINDROME --- : 32 base pairs with dyad symetry;
- TATA - : early transcription TATA box;
- cap sites - : 5' ends of early mRNAs;
xxxx : homology with SV 40 sequences;
[ ] : sequence (11 BP) deleted in A3 DNA;
MboII(A2) and -(A3): cleavage sites of MboII endonuclease in A2 and A3 DNA respectively (recognition sequence: GAAGA at nucleotide 34);
Horizontal arrows : AGAGGC and GPuGGC boxes;
Vertical arrow : left boundary of the viable deletions isolated by Bendig and co-workers (19);
ATG at 173 is the initiation codon of the three early proteins.

sequence of polyoma A3 strain lacks the 11 nucleotides from 44 to 54, removing the first AGAGGC block of A2, but maintaining the /AGAGGC/TTCC/ AGAGGC/ repeat (13,14).

A requirement for this repeated sequence for the binding of large-T to the ori region could be evidenced directly. A recognition site for the MboII endonuclease (GAAGA) is found in A2 DNA at nucleotides 34 to 38. The enzyme cleaves 8 nucleotides to the right of the 3' deoxyadenosine (20), therefore at nucleotide 46 in A2 DNA, leaving intact the sequence /AGAGGC/TTCC/AGAGGC/ between nucleotides 49 and 64. By contrast, A3 DNA will be cleaved between the two AGAGGC blocks (Figure 4). The assumption that the repeat is an essential part of the recognition site therefore leads to the prediction that binding of large-T ATPase to A3 DNA will be reduced or abolished after MboII cleavage, whereas MboII fragments from A2 DNA should bind large-T as efficiently as the uncleaved DNA. This was precisely the result observed in the experiments reported in Table 3. It was checked in all these experiments that the DNA in solution was extensively cleaved by the restriction endonuclease (data not shown).

Deletion mapping of the recognition site.

A series of plasmids containing nearly full-length polyoma virus DNA with internal deletions only in the ori region were constructed from strain A2 DNA cloned into vector pAT153 (C. Tyndall and R. Kamen, manuscript in preparation). An XhoI site, which does not normally occur in the viral or vector DNA, was inserted at nucleotide 5130 (a PvuII cleavage site), and used to obtain unique linear molecules. These were digested with Bal31 in order to produce deletions that extend for different lengths in both directions. XhoI linkers were re-introduced before circularization of the Bal31-treated linear molecules. Thus, the extent of the deletions could be accurately determined by separate double digestions with XhoI and other enzymes which cleave in nearby sequences. The limits of the deletions on the early region side were determined by restriction enzyme analysis and in some cases also by S1 analysis of hybrids formed between in vitro RNA transcripts of the deletion DNA and wild type single-stranded DNA probes. The critical deletions were more accurately positioned by DNA sequencing. We also include in this series a plasmid (pdl2020P) which lacks the small PvuII fragment (nucleotides 5130 to 5264) on the late region side of the ori sequence (Figure 3).

The various plasmid DNAs were characterized for their ability to elute specifically the large-T ATPase activity from calf thymus DNA cellulose. Results presented in Figure 5 indicate that deletion of sequences between

Table 3: Binding of large-T ATPase to MboII-cleaved A2 and A3 DNA.

DNA in solution	polyoma virus strain	ATPase ACTIVITY ELUTED (*) without MboII cleavage	after MboII cleavage
pBR 322	-	0.04	ND
pPY-1	A2	0.39	0.34
pdl2020P	A2	0.29	0.31
pPY-2	A3	0.36	0.05
pPY-45	A3	0.28	0.06
pTS25E	A3	0.30	0.07

(*) average of two to three independent measurements for each DNA.

nucleotides 5130 and 30 (mutants dl2020P, dl2033, dl2088 and dl2019) do not prevent the binding of large-T. In sharp contrast, no detectable binding could be evidenced for any of the deletions that extend past nucleotide 50. Deletion dl2086 for instance, that ends at nucleotide 53, completely abolishes the binding ability. These results are consistent with the inference that the sequence /AGAGGC/TTCC/AGAGGC/ is required for the binding of the large-T protein.

DISCUSSION.

A unique sequence in the viral DNA appears to be essential for high affinity binding of polyoma virus large-T protein. This sequence includes a characteristic repeat /AGAGGC/TTCC/AGAGGC/, located (nucleotides 49 to 64) in

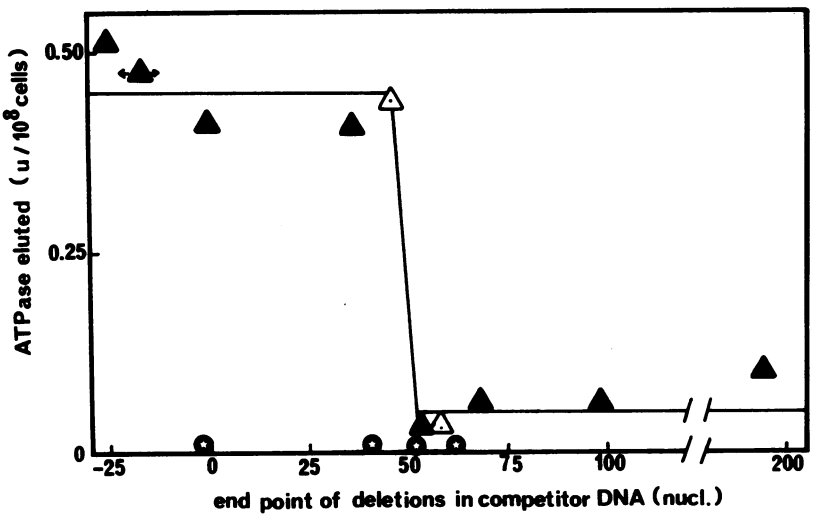

Figure 5. Localization of a primary binding site on the viral DNA for the polyoma virus large-T ATPase.

Same experiment as that described in the Legend of Figure 2, using polyoma virus DNA containing deletions centered around nucleotide 5130. Only the right boundary of each deletion is presented. Open symbols are results of the MboII cleavage experiment (Table 3), which may be assimilated to a deletion mapping point. Stars indicate the position of the AGAGGC repeats.

the general area previously defined as origin of replication (ori) (10-12). Although the protein is able to bind with a significant affinity to any double-stranded DNA structure (5), the stability of the specific complexes is several order of magnitude higher: the dissociation constant of the polyoma large-T-ori DNA complexes may be estimated at about 10^{-12} M in 0.05 M KCl.

Determination of the actual number of sites per DNA molecule occupied by large-T at saturation requires further investigation. Our present results are compatible either with only one binding site including the 49-64 repeat, or with any number of sites, provided that binding to the primary site is a prerequisite for a possible high affinity binding to the other(s). The latter possibility is made likely by the known structure of the binding site of SV40 large-T (8,9,21). It is made necessary for polyoma by the observation (Table 3) that MboII cleavage at nucleotide 46 in A2 strain DNA does not interfere significantly with the binding of large-T, whereas cleavage 11 nucleotides to the right in A3 strain DNA reduces the binding to background levels.

Experiments are in progress in order to identify possible secondary sites, by comparing the dose-response curves for various deletion mutants. This approach is made technically difficult by the limited levels of ATPase activity eluted at low DNA concentrations (see Figure 3). Preliminary results indicate that deletion of sequences to the left of the primary site decreases the apparent affinity of large-T, suggesting the existence of one or two secondary sites in the immediate vicinity.

It would thus appear that the primary interaction of polyoma virus large-T antigen with viral DNA is analogous to that previously found with SV40, in that the first binding site is slightly on the early region side of the ori sequence and possible secondary sites occur in the direction of the late region. Such an interaction in the SV40 case is also involved in the other known biochemical activity of SV40 large-T protein, repression of early region transcription. The capped 5' ends of SV40 early mRNAs (22,23) lie within the T antigen binding sites. Moreover, binding at these sites has been directly shown to block the in vitro transcription of SV40 early region (24). By contrast, the high affinity binding site for polyoma large-T protein lies some 100 nucleotides upstream of the principal "Cap" sites and 70 nucleotides before the associated "TATA" box (see Figure 4). From the data presented here, only secondary binding sites can occur in the vicinity of the "Cap" sites. In this regard, it is striking that the "Cap" sites are within a sequence homologous to the major large-T protein binding site, but in the opposite orientation (see Figure 4): four repeats of GPuGGC, located 3, 4 and 5 nucleotides apart, are present between the "TATA" box (nucleotide 120) and the translational initiation codon at nucleotide 173.

These "GGC boxes" (sequences of the general type GPuGGC) are not repeated within 20 nucleotides or less in the coding sequences of either polyoma or SV40 or BK viruses. In contrast, they are far more frequent than the statistics would predict in the major non-coding region, between the late and the early initiation codons: 8, 6 and 11 such boxes are found in SV40, BK and polyoma genomes respectively, over 461 to 625 base pairs. One of these repeats, with a pyrimidine linker on the same strand, constitutes part of the primary binding sites for SV40 (/GGAGGC/T_6/GGAGGC/) (8) and polyoma large-T proteins (/AGAGGC/TTCC/AGAGGC/, this report). A similar sequence (/GGAGGC/CT_5/TGAGGC/) is observed in BKV DNA between nucleotides 5096 and 5113 (25). It is tempting to speculate that this sequence is that recognized by BKV large-T protein. Its close similarity with SV40 site I would be in agrement with the observed complementation between BKV and SV40 early functions (26). These

sequences are different enough from the polyoma site to account for the fact that polyoma and SV 40 large-T proteins show neither cross-complementation *in vivo* (27), nor cross-recognition of their binding sites *in vitro* (see Table 2).

Such sequences are obviously not restricted to the large-T binding sites of polyomaviruses. PuGPuGGC repeats are for instance constantly observed in the mammalian (28) and avian (P. Chambon, personal communication) "Alu type" sequences, whereas an AGCAGGC box is repeated in the region of SV 40 DNA that was recently observed by Benoist and Chambon (29) to be required for the initiation of transcription. These "GGC boxes" might thus be used as basic words in the protein recognition sites of eukaryotic genomes.

ACKNOWLEDGMENTS.

We thank P. Clertant for helpful discussions. The participation in the experimental work of F. Tillier is gratefully aknowledged, as well as the generous gift of various plasmids by K. O'Hare, R. Zorob and T. Soussi. We thank L. Carbone and M.L. Varani for expert technical assistance.

This work was made possible by grants from the Délégation Générale à la Recherche Scientifique et Technique (France), the Institut National de la Santé et de la Recherche Médicale and the Centre National de la Recherche Scientifique.

REFERENCES.

1. Ito, Y., Spurr, N. and Dulbecco, R. (1977) Proc. Natl. Acad. Sci. USA, 74, 1259-1263.
2. Francke, B. and Eckhart, W. (1973) Virology, 55, 125-135.
3. Clertant, P. and Cuzin, F. (1980) Nucl. Acids Res., 8, 4377-4392.
4. Cogen, B. (1978) Virology, 85, 222-230.
5. Gaudray, P., Clertant, P. and Cuzin, F. (1980) Eur.J.Biochem., 109, 553-560.
6. Gilbert, W. and Müller-Hill, B. (1970) in The Lactose Operon, Beckwith, J.R and Zipser, D. Eds., p. 93-109, Cold Spring Harbor Laboratory.
7. Ptashne, M. (1978) in The Operon, Miller, J.H. and Reznikoff, W.S. Eds, p. 325-343, Cold Spring Harbor Laboratory.
8. Tjian, R. (1978) Cell, 13, 165-179.
9. Tjian, R. (1979) Cold Spring Harbor Symp.Quant.Biol., 43, 655-662.
10. Crawford, L.V., Syrett, C. and Wilde, A. (1973) J.Gen.Virol., 21, 515-521.
11. Crawford, L.V., Robbins, A.K. and Nicklin, P.M. (1974) J.Gen.Virol., 25, 133-138.
12. Griffin, B.E., Fried, M. and Cowie, A. (1974) Proc.Natl.Acad.Sci.USA, 71, 2077-2081.
13. Soeda, E., Arrand, J.R., Smolar, N., Walsh, J.E. and Griffin, B.E. (1980) Nature, 283, 445-453.
14. Friedmann, T., Esty, A., La Porte, P. and Deininger, P. (1979) Cell, 17, 715-724.
15. Gache, C., Rossi, B. and Lazdunski, M. (1976) Eur.J.Biochem., 65, 293-306.
16. Sutcliffe, J.G. and Ausubel, F.M. (1978) in Genetic Engineering, A.M. Chakrabary Ed., p. 83-112, CRC Press Inc.

17.De Haseth, P.L., Gross, C.A., Burgess, R.R. and Record, M.T. (1977) Biochemistry, 16, 4777-4782.
18.Myers, R.M. and Tjian, R. (1980) Proc.Natl.Acad.Sci.USA, 77, 6491-6495.
19.Bendig, M.M., Thomas, T. and Folk, W.R. (1980) Cell, 20, 401-409.
20.Roberts, R.J. (1981) Nucl.Acids Res., 9, r63-r80.
21.Mc Kay, R.L. and Di Maio, D. (1981) Nature, 289, 810-813.
22.Reddy, V.B., Ghosh, P.K., Lebowitz, P., Piatak, M. and Weissman, S.M. (1979) J.Virol., 30, 279-296.
23.Haegeman, G. and Fiers, W. (1980) J.Virol., 35, 955-961.
24.Rio, D., Robbins, A., Myers, R. and Tjian, R. (1980) Proc.Natl.Acad.Sci. USA, 77, 5706-5710.
25.Seif, I., Dahr, R. and Khoury, G. (1979) Cell, 18, 963-977.
26.Mason, D.H. Jr and Takemoto, K.K. (1976) J.Virol. 17, 1060-1062.
27.Tooze, J. (1980) The Molecular Biology of Tumour Viruses, Part II, 2nd edn. Cold Spring Harbor Laboratory, NY.
28.Jelinek, W.R., Toomey, T.P., Leinwand, L., Duncan, C.H., Biro, P.A., Choudary, P.V., Weissman, S.M. Rubin, C.M., Houck, C.M., Deininger, P.L. and Schmid, C.W. (1980) Proc.Natl.Acad.Sci.USA, 77, 1398-1402.
29.Benoist, C. and Chambon, P. (1981) Nature, 290, 304-310.
30.Eckhart, W. (1969) Virology, 38, 120-125.
31.Bendig, M.M., Thomas, T. and Folk, W.R. (1980) J.Virol., 33, 1215-1220.