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Binding of an Analog of the Simian Virus 40 T Antigen to Wild-type and Mutant Viral Replication Origins

DANIEL G. TENEN, LORA L. HAINES AND DAVID M. LIVINGSTON

The Sidney Farber Cancer Institute and
The Departments of Medicine, Brigham and Women's Hospital and the Harvard Medical School, Boston, Mass. 02115, U.S.A.

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DNAase footprint analyses of purified AD2+D2 (D2) T protein binding to simian virus 40 origin region fragments revealed a series of four specific interactions with contiguous sequences constituting a 120 base-pair block, in keeping with previous DNAase protection results reported by others. Protection was observed to extend from a 30 base-pair strong affinity site located on the early side of the replication origin (site 1) to two adjacent lower affinity sites, including the origin of replication (site 2) and a 11 to 13 base-pair site between site 1 and the beginning of the T/t coding sequence (site 1′). A fourth site (site 3) was noted abutting the late border of site 2. Binding to site 1 was associated with enhanced D2T binding to sites 2 and 1′. Thus, binding to these sites is co-operative and/or the sequences which constitute site 1 affect the conformation of the sites 1′ and 2 sequences such that they now serve as sites of more efficient D2T binding. In addition, while deletion of all of site 2 and its substitution by late viral sequences ablated processive T binding to sequences abutting site 1 on its late side, various site 2 deletions comprising up to approximately 40% of that sequence did not affect binding to that site to a major degree. Therefore, binding to the replication origin is a sequence-specific event, but there may be multiple strong protein contact sites within that sequence.

1. Introduction

The simian virus 40 (SV40) large T antigen (T) is an 81×10^3 M_r phosphoprotein which can bind specifically to sequences at and immediately vicinal to the viral origin of replication and the major cap sites for the ends of SV40 early RNA (Reed *et al.*, 1975; Jessel *et al.*, 1976; Tijan, 1978; Shalloway *et al.*, 1980; Reiser *et al.*, 1980; McKay, 1981; McKay & DiMaio, 1981; Myers *et al.*, 1981*a*,*b*). T binding to sequences within this DNA segment leads to repression of early messenger RNA synthesis (Rio *et al.*, 1980; Myers *et al.*, 1981*a*,*b*; Hansen *et al.*, 1982) and the initiation of rounds of viral DNA replication (Tegtmeyer, 1972; Chou, *et al.*, 1974; Shortle *et al.*, 1979). Previous studies have defined distinct, non-overlapping T antigen binding sites of different affinity in this region of the viral genome (Tjian, 1978; Shalloway *et al.*, 1980; Myers *et al.*, 1981*a*,*b*; McKay, 1981; McKay & DiMaio, 1981). In this paper, we have used an immunoprecipitation DNA binding assay

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(McKay, 1981) and the DNAase "footprinting" technique (Galas & Schmitz, 1978) with wild-type SV40 DNA and various deletion mutants to map these sites in detail and to begin to investigate the mechanism of binding of a closely related analog of T, the D2 hybrid T antigen (Hassel *et al.*, 1978; Tjian, 1978*a*), to these various sites. Four distinct D2T antigen-binding loci were noted, and binding to the highest affinity site was associated with facilitation of D2T binding to two adjacent lower affinity sites, one of which likely comprises the replication initiation sequence (Gutai & Nathans, 1978; Subramanian & Shenk, 1978; Shortle *et al.*, 1979; DiMaio & Nathans, 1980). Thus, it is possible that binding of the protein to three of these sites operates, in part, by a co-operative mechanism. In addition, results to be presented here indicate that for T to process from the highest affinity site to the replication origin sequence, some, but not all, of this sequence must be present. Thus, T binding to the origin is a sequence-dependent event, and there may be multiple strong protein contact sites within this genomic segment.

2. Materials and Methods

(a) *Cells and viruses*

HeLa cells were grown in suspension at 37°C in DME medium (Dulbecco's modified Eagle's medium) supplemented with 10% (v/v) horse serum (GIBCO). CV1-P monkey cells were grown in DME medium containing 10% (v/v) calf serum (GIBCO). All cells were grown in a humidified, 10% (v/v) CO_2-containing atmosphere.

Plaque-purified SV40, strain 776, and Ad2+D2 virus (kindly provided by Dr J. Sambrook) were grown in CV1-P cells as described previously (Jessel *et al.*, 1976; Hassel *et al.*, 1978). SV40 mutants cs1085 and cs1088 (DiMaio & Nathans, 1982) were generously provided by Drs D. DiMaio and D. Nathans.

(b) *Cloned DNA*

Wild-type SV40 DNA and origin mutant DNAs 1–11, 6–17, and 8–11 (Gluzman *et al.*, 1980*a*), cloned in the plasmid pMK16, were a gift from Dr J. Gluzman. They were propagated in the *dam*$^-$ strain RB404 (a gift from Dr Roger Brent).

(c) *Viral DNA*

For preparation of wild-type SV40 strain 776 or mutant cs1085 and cs1088 viral DNAs, 90% confluent cultures of CV1-P cells were infected at a multiplicity of 10. After 48 h at 37°C, the media was removed and replaced with 0·03 ml/cm^2 of 10 mM-Tris·HCl (pH 7·4), 10 mM-EDTA, 0·6% (w/v) sodium dodecyl sulfate (Hirt, 1967). After 15 min at 37°C, lysates were collected from each plate, and 0·2 vol. 5 M-cesium chloride was added. After gentle inversion, the mixture was incubated at 4°C for 16 h. Cellular DNA was pelleted by centrifugation for 1 h at 15,000 revs/min in a Beckman JA20 rotor in a J21B centrifuge. Solid cesium chloride was added to the supernatant to a refractive index of 1·3885. After addition of 0·005 vol. ethidium bromide (10 mg/ml), the mixture was centrifuged for 24 h in a Beckman Vti50 rotor at 42,000 revs/min. The isolated form I band was then rebanded in a $CsCl_2$/ethidium bromide gradient under similar conditions. The ratio of absorbance at 260 nm/280 nm was $\geq$ 1·98 for all DNAs prepared in this manner. This method was developed and suggested to us by Dr J. Tevethia.

Viral and cloned mutant DNAs were cleaved with *Pvu*II, *Taq*I, *Hin*fI, *Bst*NI, *Bgl*1, *Msp*I, and *Alu*I restriction endonucleases (New England Biolabs) according to protocols recommended by the manufacturer.