LOCATION OF THE T4 GENE 32 PROTEIN BINDING SITE ON POLYOMA VIRUS DNA

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1. Introduction

Incubation of superhelical DNA with the bacteriophage T4 gene 32 protein followed by glutaraldehyde fixation yields a circular structure with a small denaturation loop that can be visualized by electron microscopy [1]. Studying SV 40 DNA, Morrow and Berg [2,3] showed that the denaturation loop is located in a specific region (0.45 genome length) by cleavage with either one of the Eco-R1 and HpaII restriction enzymes that produce unit length linears of SV 40 DNA. In a previous study, we reported (4) the presence of a single T4 gene 32 protein denaturation loop on polyoma virus superhelical DNA and its location relative to the unique Eco-R1 cleavage site. The major binding site was located at 0.22 (± 0.02) genome lenght from the nearest Eco-R1 end and two minor sites at 0.09 (± 0.03) and 0.41 (± 0.01). Because the two ends of the Eco-R1 generated linear molecules are indistinguishable, an ambiguity remained as to the absolute location of the T4 gene 32 protein major binding site(s) on the polyoma DNA physical map established by Griffin et al. [5]. An alkaline denaturation map of polyoma (Py) DNA recently established in our laboratory [6] showed the presence of two A-T rich regions at about 0.2 and 0.8 of the polyoma DNA molecule, either of these sites or both can bind the gene 32 protein in the superhelical molecule.

In an attempt to resolve this ambiguity, we searched for another restriction endonuclease that will cleave PyDNA in a unique site.

2. Materials and methods

Superhelical polyoma DNA was isolated from cells infected with plaque purified LP polyoma virus. Digestion of this DNA with the HpaII restriction endonuclease gave eight fragments identical in mobility to those described by Griffin et al. [5] for the A2 isolate of this virus. Eco-R1 restriction endonuclease was purified according to the procedure of Yoshimori [7] by phosphocellulose and hydroxylapatite chromatography and was devoid of contaminating endo or exo nucleases. HpaII endo nuclease was prepared according to Sharp et al. [8], and HindII, III endonucleases according to Smith and Kelly [9]. BamI restriction endonuclease was purified according to Wilson and Young [10] and was a gift of M. Perricoudet. Bacteriophage T4 gene 32 protein was purified from T4 gene 55 *am* infected *E. coli* B cells, according to Alberts and Frey [11] with a final step of column chromatography on hydroxyapatite [1]. Samples of gene 32 protein Py DNA were prepared and examined by electron microscopy as previously described [4]. Gradient polyacrylamide gel electrophoresis of DNA fragments were done according to Jeppeson [12].

3. Results and discussion

3.1. *BamI endonuclease cleaves Py DNA at a unique site (0.58).*

A restriction endonuclease (BamI) was recently

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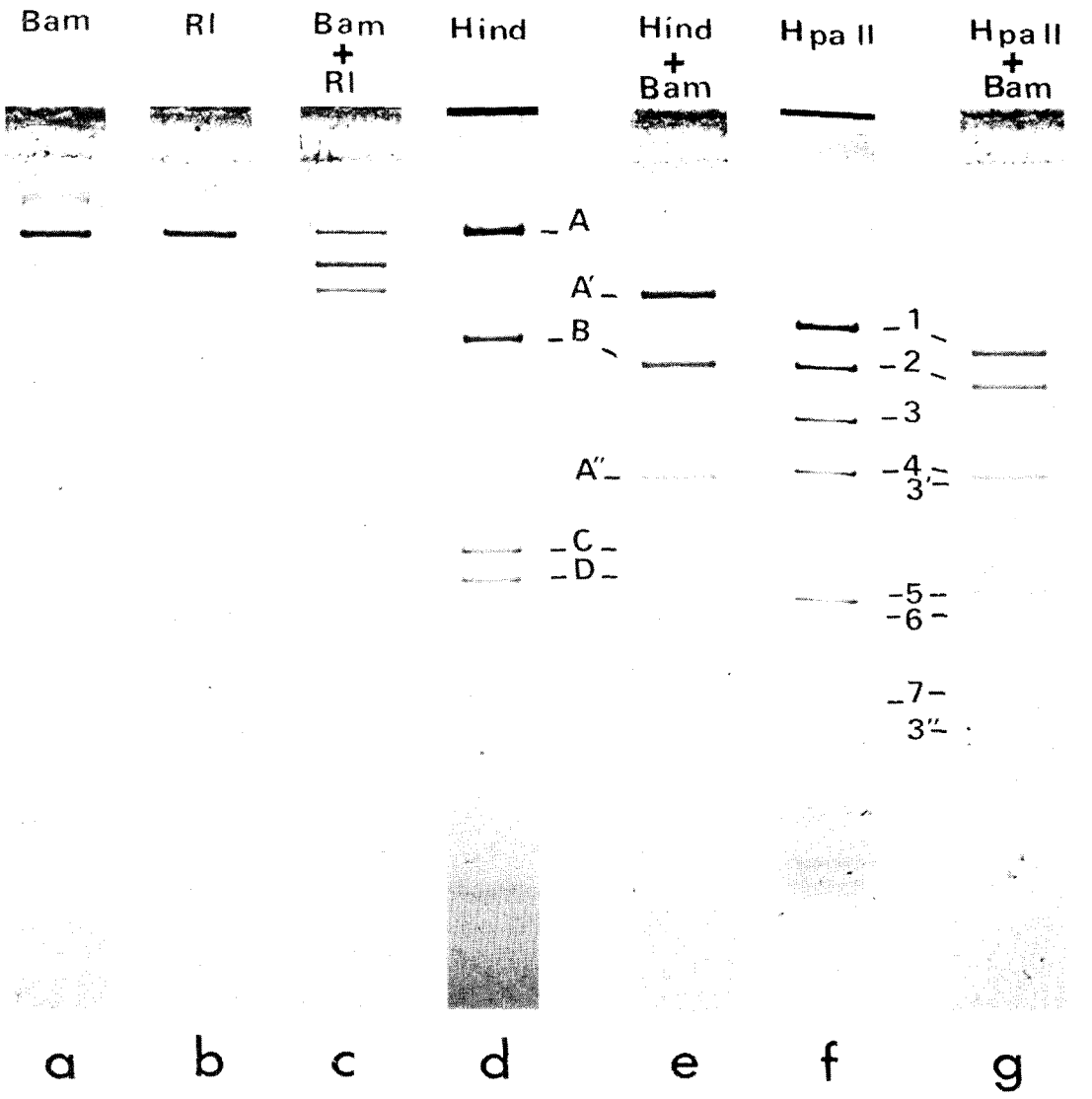

Fig.1. Polyacrylamide gel electrophoresis of restriction endonucleases cleavage products of Py DNA. Viral DNA (1–2 μg) was digested with the following endonucleases: (a) BamI, (b) Eco-R1, (c) Eco-R1 + BamI, (d) HindII, III, (e) HindII, III + BamI, (f) HpaII, (g) HpaII + BamI. All the restriction enzymes, except BamI, were used in a 2–5–fold excess to ensure complete digestion. This explains the lack of total cleavage in the BamI site. The DNA fragments were isolated after phenol extraction, ethanol precipitated and electrophoresed for 10 hr on a 2.5% to 10% (a,b,c,e,g) or 2.5% to 13.5% (d,f) polyacrylamide gradient slab gels [12]. The DNA bands were stained with methylene blue.

isolated from *Bacillus Amyloliquefaciens* [10]. Treatment of Py DNA with this enzyme generated full length linears that comigrated with Eco–R1 linears of Py DNA on polyacrylamide gels (fig.1). Localisation of the BamI cleavage site relative to the Eco-R1 site [4,13,14] was achieved by cleaving the superhelical DNA with a mixture of both enzymes. As shown in fig.1 (e) two fragments were produced with an approximate length of 1.8×10^6 and 1.35×10^6 daltons. When spread by the cytochrome *c* Formamide technique for electron microscopy observation, the fragments length was 0.41 ± 0.02 and 0.59 ± 0.03 Py

fractional length. The BamI site is thus located at about 0.41 fractional length to the right or to the left of the Eco-R1 site. Absolute location of the BamI site was achieved by digestion of Py DNA with HindII, III or HpaII in the absence or presence of BamI (fig.1). Comparison of the digestion patterns show that BamI cleaved the HindII, III A fragment [15] and the HpaII fragment 3 [5]. The length of the new fragments produced was calculated from the migration relative to known fragments of Py DNA. BamI cleaved at a distance of 0.132 Py fractional length from the A,D border [15] and at a distance of 0.043 Py fractional length from the 3,1 border [5]. These results confirm the electron microscopy measurements and locate the BamI cleavage site at position 0.582 ± 0.003 of the physical map of Py DNA. The cleavage site of BamI on Py DNA was independently localised by M. Fried (personal communication).

3.2. *Location of bacteriophage T4 gene 32 protein binding sites.*

Polyoma superhelical DNA was treated with the T4 gene 32 protein and fixed with glutaraldehyde as previously described [4]. After dialysis, the DNA samples were treated with either Eco-R1 or BamI restriction enzymes and the resulting products were spread in the presence of cytochrome C and Formamide [16]. The distance between the middle of the denaturation loops and the nearest Eco-R1 or BamI end was measured and the histograms are given in fig.2. In agreement with our previous observations [4], the majority of the denaturation loops (70%) were located in a broad distribution at position 0.23 ± 0.2 from the Eco-R1 end (fig.2a). The distribution of the loop position relative to the nearest BamI end (fig.2b) shows two major peaks in positions 0.34 ± 0.02 and 0.22 ± 0.02. The major peak seen on the Eco-R1 linears is composed effectively of two sites, one at position of 0.24 (0.34 on BamI) and the other at position 0.80 (0.22 on BamI) of the Py genome. The frequency of loops in these sites was 42% (0.24) and 29% (0.80) of the total loops observed.

About 30% of the loops observed are distributed between several minor binding sites. Comparison of the Eco-R1 and BamI distributions suggests the presence of minor sites at the following positions on the Py genome: *a.* position 0.08 (0.49 on BamI, 0.08 on Eco-R1); *b.* the Eco-R1 site at position 0.42 ± 0.03 is

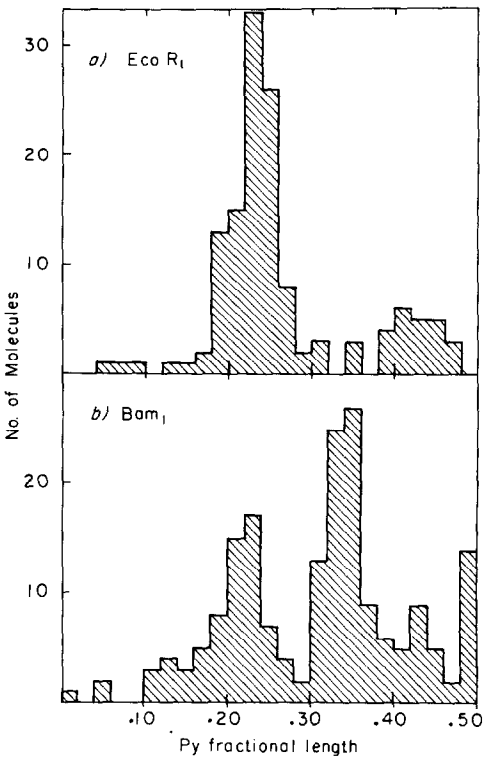

Fig.2. Histograms of the distance between the middle of the T4 gene 32 protein denaturation loop on Py DNA and the nearest end of the linear DNA. (a) cleavage with Eco-R1; (b) cleavage with BamI.

composed of a site at position 0.45 (0.13 on BamI) and a site at position 0.59, these later molecules are not cleaved by BamI; *c.* the minor peak at position 0.42 ± 0.02 on the BamI histogram indicates the possible presence of loops in the origin of the physical map, these loops are not cleaved by Eco-R1. Monjardino and Cowie [17] mapped the gene 32 protein binding sites on Py A2 virus DNA by comparing the Eco-R1 linea with the two fragments produced by HindIII cleavage. They observed binding sites in similar positions with exception of the site near the Eco-R1 or HindIII cleavage site. However, the two major sites that they observed were in positions 0.23 and 0.56 of the Py map.

In conclusion, we showed that the superhelical Py DNA can be alternatively denatured at either one of the A-T rich regions. The two major sites that bind

gene 32 protein are in positions 0.24 (42%) and 0.80 (29%). Minor binding sites are located in positions zero, 0.08, 0.45 and 0.59 of the Py physical map [5].

All the regions that bind gene 32 protein on Py DNA are relatively rich in A-T base pairs as shown by the alkaline denaturation studies [6]. Recent studies on PM2 DNA showed that the 7 possible locations of gene 32 protein binding sites are located in A-T rich regions (C. Brack, personal communication).

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