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

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SUMMARY

Three easily denatured regions can be demonstrated in polyoma virus DNA. T4 gene 32 protein which binds to single stranded DNA, but not to duplex DNA, will specifically bind to any of these sites when viral DNA is in its superhelical configuration. These sites were mapped relative to a unique *E.coli* R_I endonuclease cleavage site by electron microscopy.

Bacteriophage T4 gene 32 protein, an unwinding protein which specifically binds to single stranded DNA (1) has been shown by Morrow and Berg (2,3) to bind at a unique site to SV 40 virus superhelical DNA. This site is likely to correspond to an A-T rich region where the two strands are locally melted in a superhelical molecule. Uniqueness of this site on SV 40 DNA makes it usefull as a marker for physical mapping of the viral genome. We applied a similar approach to the study of polyoma virus DNA.

MATERIAL AND METHODS

1) Cells and virus : preparation of polyoma DNA.

Conditions for cells and virus growth, and procedure for purification of polyoma DNA have been previously described (4).

2) Purification of Eco R_I restriction endonuclease.

The enzyme was purified from *Escherichia coli* strain RY 13,

according to the procedure of Yoshimori (5), up to a point where no contaminant endonuclease activity could be detected (as checked by the conversion into form II of bacteriophage PM 2 component I DNA, which lacks the Eco R_I site (6)).

3) Purification of T4 gene 32 protein.

The protein was purified from T4 gene 55 am infected *E.coli* B cells, according to Alberts and Frey (1) with a final step of column chromatography on hydroxyapatite (7).

4) Binding of T4 gene 32 protein to polyoma DNA component I and fixation of the complex.

The conditions described by Delius et al. (7) have been employed. After glutaraldehyde fixation, the samples were dialysed, treated by Eco R_I and spread for electron microscopy.

5) Electron microscopy.

Samples were mounted on paralodium coated copper grids for electron microscopy by the formamide technique (8). Pt-Pd shadowed grids were examined and photographed with a Siemens Elmiskop 101. DNA molecules were measured using a laboratory made coordinatometer connected to a PDP-8 Digital Computer.

RESULTS AND DISCUSSION

Eco R_I restriction enzyme introduces only one break in polyoma DNA, producing unit length linear molecules (2). Uniqueness of the cleavage site was established by denaturing in 50% formamide, and renaturing the linear product according to the procedure of Morrow and Berg (2). Most of the renatured duplexes were linear, indicating that they did not correspond to circular permutations of viral DNA sequences. Another confirmatio

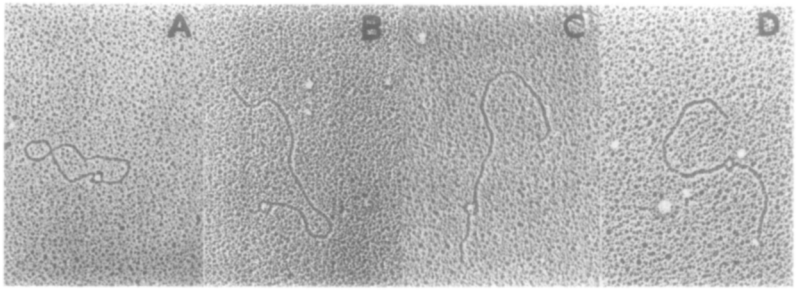

Figure 1 : Electron micrographs of polyoma form I DNA complexed to T4 gene 32 protein (A), and of polyoma DNA-T4 gene 32 protein complexes treated with Eco R_I endonuclease (B,C,D).
Magnification 32,000 x

for uniqueness of the Eco R_I cleavage site comes from the results of Crawford et al. (9) who localized the origin of replication at 0.29 genome length from the cleavage site.

After binding of T4 gene 32 protein to polyoma DNA and glutaraldehyde fixation as described under Methods, the resulting complexes were examined by electron microscopy. They are seen as open circular structures with one denaturation loop per molecule (fig. 1 A). No molecules with more than one denaturation loop were found ; this result is likely to be due to closing of second and third sites by binding at the first site. In the presence of Eco R_I, the complex is cleaved once , giving rise to three classes of molecules of the same total length (equal to that of the circular complex), but with the denatured region at different distances from the nearer end of the molecule (fig. 1 B, C, D). These distances were measured on 165 molecules, and the distribution of these values, as well as that of the total length of the molecules are shown in fig. 2. Three classes of linear complexes are evident, the loop being located at 0.09 (± 0.03), 0.22 (± 0.02) or 0.41 (± 0.01) genome length from the nearer end

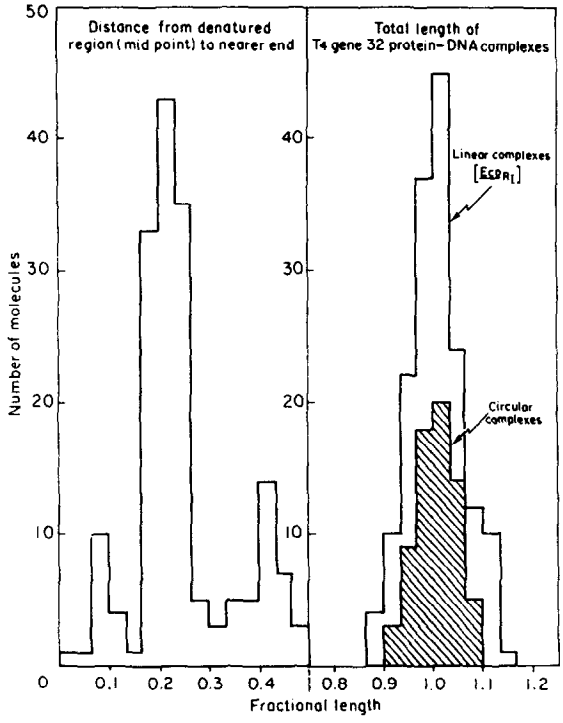

Figure 2 : Histograms of length measurements of circular and R_I linear polyoma DNA-T4 gene 32 protein complexes, and of distances from the "denaturation loop" to the nearer end, as a fraction of the total length of the complex (= 1.83 ± 0.02 μ (mean ± error of the mean)).

(mean ± error of the mean (P = 0.05)). Respectively 12, 62 and 26% of the molecules were found in each class.

The localization of the 3 binding sites relative to each other and their absolute location relative to the Eco R_I site cannot be deduced from these data. Each binding site can be placed at either one of two symetrical positions relative to the Eco R_I site on the circular polyoma DNA map. Further studies using other references sites for an absolute determination are in progress.

The number of binding sites on polyoma DNA apparently differs from that of SV 40. A close examination of the published data (2), however, reveals the existence of minor binding sites,

which we have also found in similar experiments using SV 40 DNA (our unpublished results).

It can be concluded that in a non-defective Eco R_I sensitive polyoma DNA molecule, there are 3 discrete regions of weak hydrogen bonding, which are likely to correspond to AT rich regions. This conclusion is in agreement with a study by Germond, Vogt and Hirt (10) on the sites at which the single strand specific S1 endonuclease cleaves polyoma DNA.

Such discrete AT rich regions are certainly usefull as markers for establishing a physical map of the virus genome. They may play a role in the recognition of the DNA molecule by various proteins.

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