

NOTES

Denaturation Map of Polyoma DNA

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A denaturation map of polyoma DNA cleaved by *Eco* R₁ to form linear molecules was established by electron microscopy. Partial denaturation, under the same conditions, of fragments obtained by *Haemophilus influenzae* restriction enzymes allowed us to align the denaturation map with the already established physical map of polyoma DNA (Griffin et al., 1974).

Polyoma virus DNA consists of double-stranded circular molecules. Physical mapping of the viral DNA is important for localizing functionally important regions of the genome (recognition sites of various proteins, early and late genes, etc.). A map has been recently published using specific fragments produced by restriction enzymes. Localization of the A-T- or G-C-rich regions of the molecules could be independently obtained by partial denaturation mapping. These two maps could be superimposed by establishing the denaturation pattern of isolated fragments obtained after cleavage by restriction enzymes.

In previous studies (1, 8), partial denaturation of relaxed circular polyoma DNA was studied. The absence of a reference point on circular DNA caused difficulties in the interpretation of the results. *Eco* R₁ (*Escherichia coli* R₁) restriction enzyme introduces one double-strand break (11) at a unique site (4, 13) in polyoma DNA. The linear molecules were partially denatured by alkaline treatment (10) and then spread for electron microscopy in the presence of cytochrome *c* by the formamide technique (5). Figure 1A shows two partially denatured molecules examined by electron microscopy. All the molecules of unit length that were observed showed a common structure: one of the ends was denatured whereas the other end was native. This property helped to orient partially denatured molecules relative to each other without ambiguity. The size and distance to the end of the denaturation loops were thus measured on 50 molecules (Fig. 2A). The histogram of the native regions derived from the denaturation maps of Fig. 2A is shown in Fig. 3A. At pH 11, used for partial denaturation, three major native regions appeared to be lo-

cated at, respectively, 0.42, 0.71, and 0.90 to 1.00 fractional lengths from the left-hand (denatured) end of the molecules, and three minor native regions appeared to be located at, respectively, 0.09, 0.27, and 0.63. These regions are presumably rich in G-C base pairs. Four main regions, which denatured readily and were probably rich in A-T base pairs, were located at, respectively, 0 to 0.07, 0.13 to 0.24, 0.52 to 0.58, and 0.78 to 0.86 from the left-hand end of the molecules. Further experiments were necessary to correlate the linear denaturation map with the physical map of the *Hpa* II (*Haemophilus parainfluenzae*) fragments of polyoma DNA recently established (9). To solve this problem, we studied the denaturation of fragments produced by cleavage with *Hin* (*Haemophilus influenzae*) restriction enzymes. *Hin* III enzyme introduces two breaks in polyoma DNA at, respectively, 0.015 and 0.455 genome lengths from the *Eco* R₁ site to produce two fragments (9). *Hin* II enzyme introduces two breaks in polyoma DNA at, respectively, 0.26 and 0.36 genome lengths (2, 7; our unpublished observations). Thus, the simultaneous digestion of polyoma DNA by *Hin* II and *Hin* III gives four fragments: a major fragment of 0.56 fractional length of the molecule and three minor fragments (0.25, 0.098, and 0.087 fractional lengths). The mixture of the *Hin* fragments was used for partial alkaline denaturation under the conditions described above for *Eco* R₁ linear DNA. Fig. 1B shows two partially denatured major molecular fragments. Twenty-five molecules were scanned and measured for denaturation loops (Fig. 2B). The histogram of the native regions derived from the individual denaturation maps of Fig. 2B is presented in Fig. 3B. These results clearly show that the histogram of

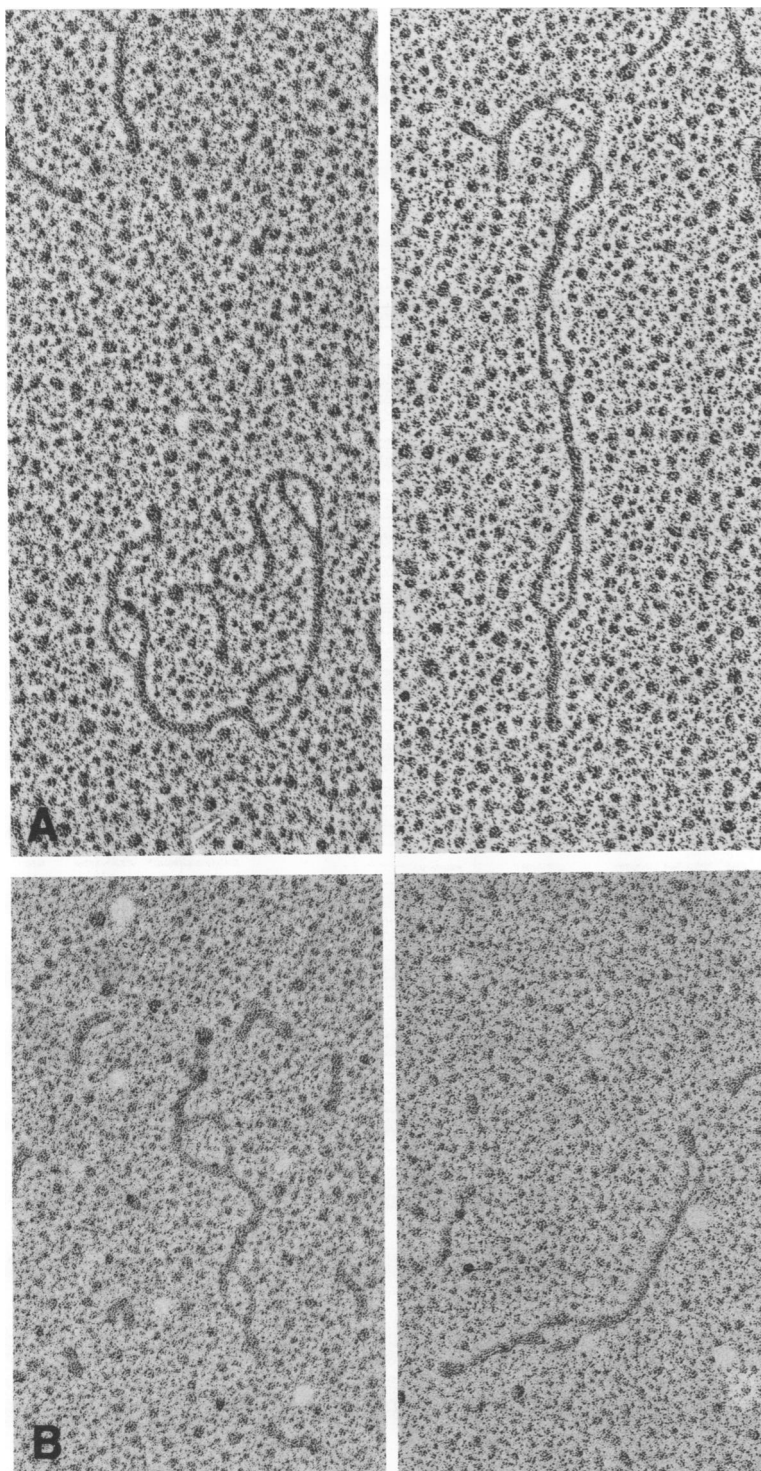


FIG. 1. Visualization of partially denatured molecules. Electron microscopy of two partially denatured *Eco R₁* linear polyoma DNA molecules (A) and of two *Hin II* and *III* partially denatured major fragments of polyoma (B). Polyoma DNA was extracted from primary mouse kidney cells cultures infected at 40 PFU/cell with virus derived from recent plaque isolates. The digestion pattern of this DNA by *Hpa II* is similar to that described by Griffin et al. (9). Polyoma DNA (0.2 μ g) from *Eco R₁* or *Hin II* and *III* digestion was incubated in 8 μ l of a solution containing 30 mM Na_2CO_3 , 6 mM EDTA, and 10% of formaldehyde, previously adjusted to pH 11 with NaOH. After 15 min, the incubation mixture was diluted to a volume of 0.04 ml and to a final concentration of 0.1 M Tris-hydrochloride, pH 7.5, 10 mM EDTA, and 50% formamide, and then spread in the presence of cytochrome c on a hypophase of 17% formamide. Grids were picked and rotary shadowed with Pt.Pd. They were examined and photographed with a Siemens Elmiskop 101 microscope at a magnification of 16,000. DNA molecules were measured on photographic enlargements, using a laboratory-made coordinatometer connected to a PDP-8 digital computer.

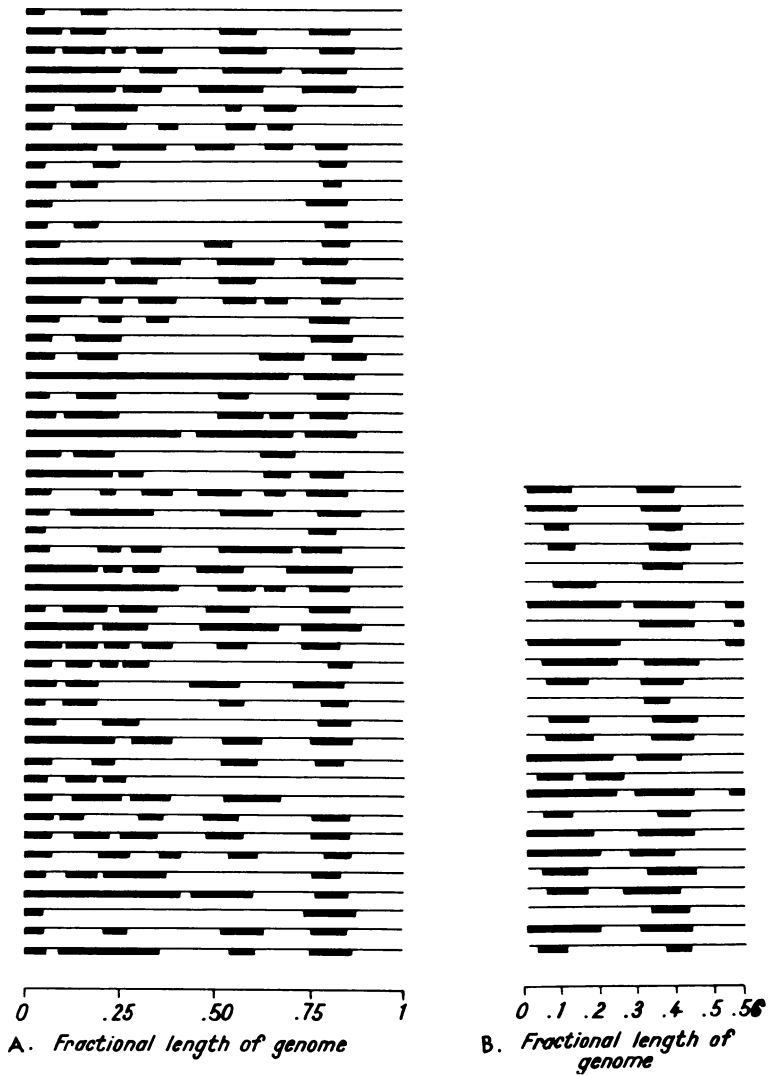


FIG. 2. (A) Alkaline denaturation maps of *Eco R*₁ linear polyoma DNA. Black areas represent the denatured part of the molecules. The unit length scale represents the average length, $1.83 \pm 0.09 \mu\text{m}$. (B) Alkaline denaturation maps of 25 *Hin II* and *III* major fragments of polyoma DNA. The unit scale length represents the average length, $1.03 \pm 0.06 \mu\text{m}$.

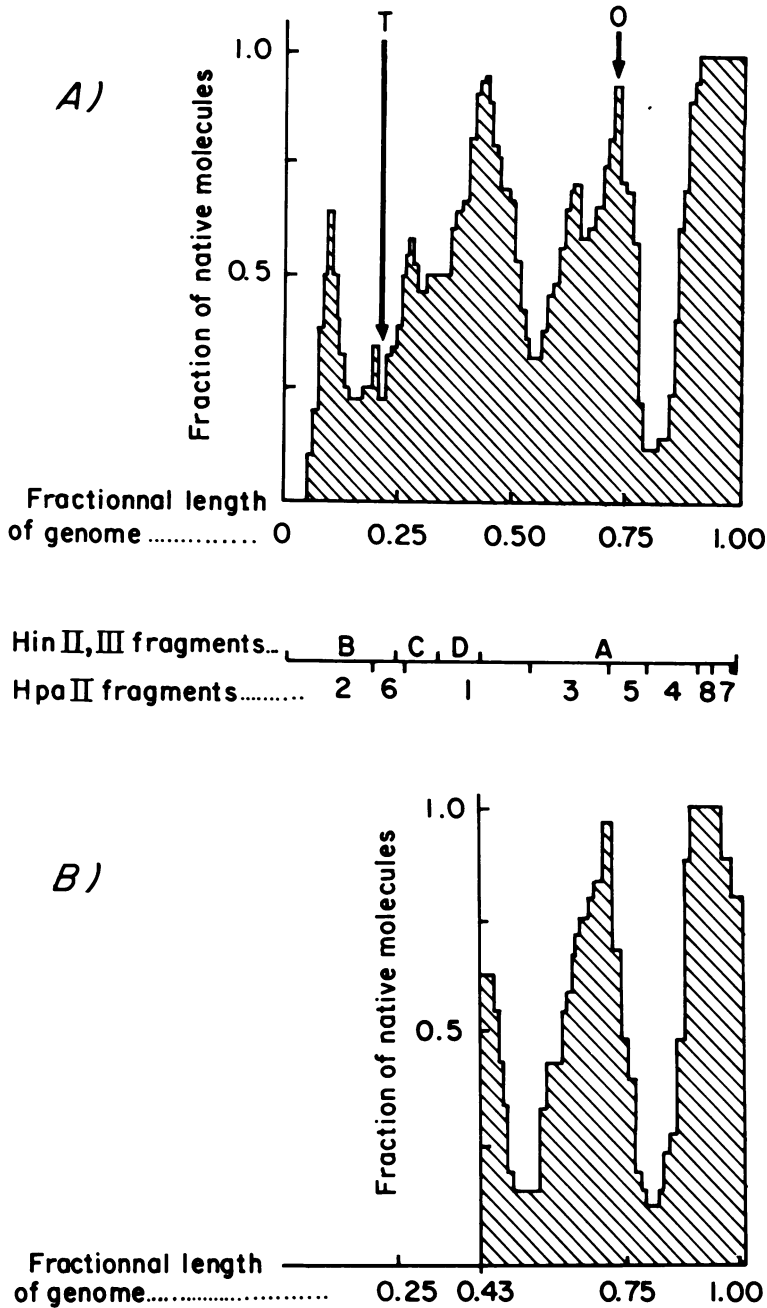


FIG. 3. Histogram of native regions. (A) Histogram of the native regions in the *Eco* R₁ linear partially denatured DNA derived from the maps shown in Fig. 2A. Arrows show the origin (O) and the termination (T) sites of polyoma replication. (B) Histogram of the native regions in the *Hin* II and III partially denatured major fragments from the maps shown in Fig. 2B. The cleavage sites of *Hpa* II and *Hin* II and III restriction enzymes on *Eco* R₁ linear polyoma DNA are represented between the two histograms.

the major fragment from *Hin* II, III digestion could be easily correlated with the right-hand (native) end of the *Eco*R₁ linear DNA histogram of denaturation (Fig. 3A). Therefore, the orientation of the denaturation map can be

unambiguously defined relative to the map of *Hpa* II fragments of Griffin et al. (9). Moreover, these results agree with the order and base composition of polyoma DNA fragments obtained by digestion with *Hpa* II (9).

It may be significant that on polyoma as well as on simian virus 40 (3, 6, 9, 12), the origin of replication is a G-C-rich region whereas termination of replication occurs in an A-T-rich region.

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