JOURNAL OF VIROLOGY, May 1976, p. 783-784

Vol. 18, No. 2
Printed in U.S.A.

NOTES

DNA-Protein Complex in Circular DNA from *Bacillus* Bacteriophage GA-1

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Received for publication 26 September 1975

DNA prepared from bacteriophage GA-1 contains circular DNA molecules, which are converted to linear molecules by treatment with trypsin.

In an earlier paper (1) we presented evidence that protein is bound to purified DNA from *Bacillus* bacteriophage GA-1. Removal of the DNA-associated protein by treatment with proteolytic enzymes results in (i) loss of transfecting activity of GA-1 DNA on competent *Bacillus subtilis* cells and (ii) an increase (0.004 g/cm^3) in the buoyant density of the DNA. In the present study we have examined the effect of trypsin treatment on GA-1 DNA by electron microscopy. GA-1 DNA was extracted from phage particles as previously described (1). The DNA was banded in a CsCl gradient, and peak fractions were pooled and dialyzed against SSC (0.15 M NaCl, 0.015 M citrate, pH 7.6). GA-1 DNA (5 μg/ml) was treated with trypsin (20 μg/ml), previously checked to be free of contaminating DNase activity. The reaction was terminated by the addition of 1% sodium dodecyl sulfate (final concentration). DNA was deproteinized with phenol and banded again in a CsCl-gradient. Control DNA without trypsin was treated similarly. Details of the experimental procedure have been published (1).

Samples of trypsin-treated and untreated GA-1 DNA were prepared for electron microscopy with a modification of the intercalating dye method (4). DNA was fixed with glutaraldehyde (Merck; 0.1% in 0.01 M Tris, 0.01 M magnesium acetate, pH 8.8) for 10 min at 37 C and quenched in ice. Ethidium bromide was added to a final concentration of 0.1 μg/ml, and 0.1-ml droplets of the DNA-ethidium bromide mixture were placed on Parafilm. After 10 min a piece of freshly cleaved mica was touched to the droplet with the concomitant adsorption of the DNA to the mica surface. A pseudoreplica of the mica was made as described by Koller et al. (4). Specimens were examined with a Philips EM 300 electron microscope, and classification of molecules was done directly on the viewing screen of the microscope.

GA-1 DNA mounted in this way appeared as 6.4-μm-long circular and linear molecules. Of 170 DNA molecules examined in the untreated GA-1 DNA preparation, 30 (18%) were found to be circular (Fig. 1). A similar value was obtained from another independently prepared DNA preparation: 45 (20%) out of 227 molecules were circles. In addition we observed linear molecules where the ends were in close (<0.05 μm) opposition, indicating breakage during mounting. No circular molecules among 500 analyzed were observed in trypsin-treated DNA. We conclude from this experiment that protein material is involved in circularization of GA-1 DNA.

When electron microscopy was done with the protein monolayer method of Kleinschmidt (3), no circular molecules (850 molecules analyzed) were observed in untreated GA-1 DNA. This indicates that the spreading forces in the formation of the protein film cause dissociation of the joined ends. We consider, therefore, the value of 20% circular molecules to be a minimum value, since dissociation of joined ends may well occur during isolation and preparation of the DNA. In untreated DNA preparations we observed a number of molecules with a small dot (Fig. 1). The frequency of dots was 58% among circular molecules and 20% among linear strands. Dots on linear strands were always positioned at one or both ends of the molecule. In trypsin-treated preparations no dots were observed.

Since the intercalating dye method does not make use of a protein monolayer, as in the classical Kleinschmidt technique, it is suitable for detection of proteins associated with DNA. Therefore we believe that the dots represent the protein linker involved in circularization of DNA. This idea is compatible with the observa-

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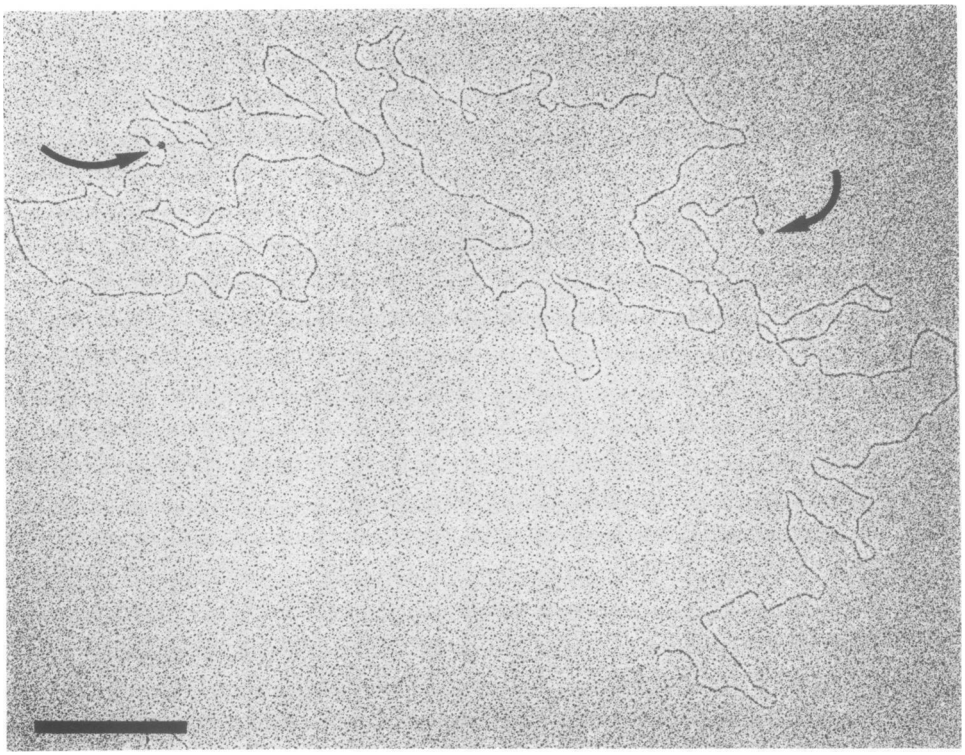

FIG. 1. *Electron micrograph of untreated GA-1 DNA showing two circular and one linear molecule. Note the dots indicated by the arrows. See text for further details. Bar, 0.5 μm.*

tion that dots occur on circular structures and on the end(s) of linear strands. The alternative explanation, that the dots represent a nonspecific protein contamination in our untreated DNA, is not ruled out. However, this explanation is hard to reconcile with the specific position of the dots on the linear DNA molecules.

Our observations on GA-1 DNA are similar to those obtained for DNA from *Bacillus* phage ϕ29 (2, 5). Ortin et al. (5) reported about 50% circular molecules for ϕ29 DNA isolated by Spherosyl chromatography after treatment of the phage with Sarkosyl at 65 C. Another possible analogy is the circular DNA-protein complex of adenoviruses (6). A biological function of the protein in the circular DNA-protein complexes is not known. Neither is there conclusive evidence for the structure of the ends of ϕ29 or GA-1 DNA. The protein linker in ϕ29 or GA-1 DNA may join the two ends of a DNA molecule that does not contain cohesive ends or alternatively may stabilize short and/or AT-rich cohesive ends of the DNA. Preliminary enzymatic evidence obtained for ϕ29 DNA (2) favors the latter hypothesis for DNA from phage ϕ29.

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