Proc. Indian natn. Sci. Acad.
Vol. **42**, Part **B**, Nos. 2 & 3, pp. 142-148
Electron Microscopy

LENGTH OF LAMBDA-NIN 5 DNA

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(Communicated by N. N. Das Gupta, FNA)

Received 19 *July* 1976

The length of osmotically released DNA from the bacteriophage lambda-nin5, a mutant of the lambda wild-type phage, was directly measured under the electron microscope. The mean observed length was $14.2 \pm 0.2\mu$, corresponding to a molecular weight of 27.3×10^6 daltons. Along with linear duplex molecules, six endless molecules were also observed.

INTRODUCTION

The functional properties of any virus are determined by the exact size and the sequence of nucleotides in its nucleic acid. For this reason, many physical techniques, e.g. light scattering, P^{32} and H^3 autoradiography, sedimentation, end labelling and electron microscopy have been utilised to acquire knowledge about the exact size of the bacteriophage genome (Thomas and MacHattie 1967). Amongst these methods, electron microscopy offers the unique opportunity of directly observing the entire DNA molecule and of measurement of its exact length. The importance of DNA length determination has been enhanced recently due to the existence of a number of mutants of the same phage with varying functional properties. These variations are caused by deletions or substitutions in the different parts of the DNA genome.

DNA from the bacteriophage lambda (wild type) has been measured in the electron microscope by Ris and Chandler (1963), MacHattie and Thomas (1964), Caro (1965), Kaiser and Inman (1965), Davis and Hyman (1971).

Very little work has been done on the direct length measurement of the DNA of mutants of bacteriophage lambda by electron microscopy. In the present work, the length of lambda-nin5 DNA was measured. It has been reported from genetic studies that bacteriophage lambda-nin5 differs genetically from lambda wild type. It has a deletion located between 83.8 and 89.2 lambda-units from the left end of the lambda wild type genome (Fiandt *et al.* 1971). The present work aims at the direct physical measurement of length of lambda-nin5 DNA.

MATERIALS AND METHODS

Bacteriophages

A concentrated stock of lambda-nin5 phage (conc. 10^{12}/ml) was the kind gift from Dr S. Palchaudhury (New York University School of Medicine). Diluted

samples (10^{10}/ml) were prepared from this in 0.1M Tris, 0.1M EDTA, *p*H 7.0, and used in different experiments.

Preparation of samples

The osmotic shock technique which was initiated by Kleinschmidt *et al.* (1962) and modified for lambda phages by Caro (1965) was used. Spreading was done as described by Davis *et al.* (1971). A mixture of 40 µl of 8M ammonium nitrate solution, *p*H 8.0, and 10 µl of 1% cytochrome C solution, *p*H 8.0 (solution made in 0.01M Tris, 0.001M EDTA) containing about 5×10^9 phages was incubated at the room temperature (22°C) for half an hour. The suspension was then spread at room temperature on the surface of 0.15M ammonium acetate solution, *p*H 8.0. The spread protein film was picked up on collodion-coated copper grids and stained in the staining solution for 2 min. The stock staining solution was 0.25M uranyl acetate in 0.05N HCl. This was diluted 500 fold in 90% ethanol prior to use.

Electron microscopy and length measurement

Electron micrographs were made with an Elmiskop 101B at an accelerating voltage of 40kV and in the magnification range 6,000 – 20,000 ×. For measurement of DNA lengths, the micrographs were projected at a final magnification of about 100,000 × on a large white sheet of board and the DNA contour traced out. This contour was measured with a map-measuring device.

Results and Discussion

Fig. 1 shows an incompletely released DNA molecule. The molecule is seen to be released through the tail of the phage (Caro 1965). Of the total number of molecules studied in this work, 50% were found to be released in this way. The released lengths in such cases varied from 3µ to 13µ. The end of the DNA farthest from the phage was always more coiled up. The phage head was dark because of accumulation of the stain within the space emptied by the released DNA. The head was found to be considerably shrunk and distorted in appearance.

Figs. 2 and 3 show two completely released molecules. Length measurement was done on 15 such molecules and the average was 14.2±0.2µ. Unlike the incompletely released molecules, the variation in the lengths of the completely released molecules was slight, as shown by the standard error.

Of particular interest in this work was the observation of endless molecules in osmotic shock preparations (Fig. 3). The average length of such molecules was 14.2±0.3µ indicating that they represented the entire DNA genome of lambda-nin 5 phage. Such circularity was observed in 25% of the molecules.

Under the assumption that DNA on electron microscopic grids retains its B crystallographic form, the molecular weight of lambda-nin5 DNA was estimated to be $27.3\pm0.5\times10^6$ daltons, if the linear density is assumed to be 192 daltons per Å (Langridge *et al.* 1960).

The length of the DNA molecule from lambda wild-type phage as estimated electron microscopically by different workers has been summarised in the following table.

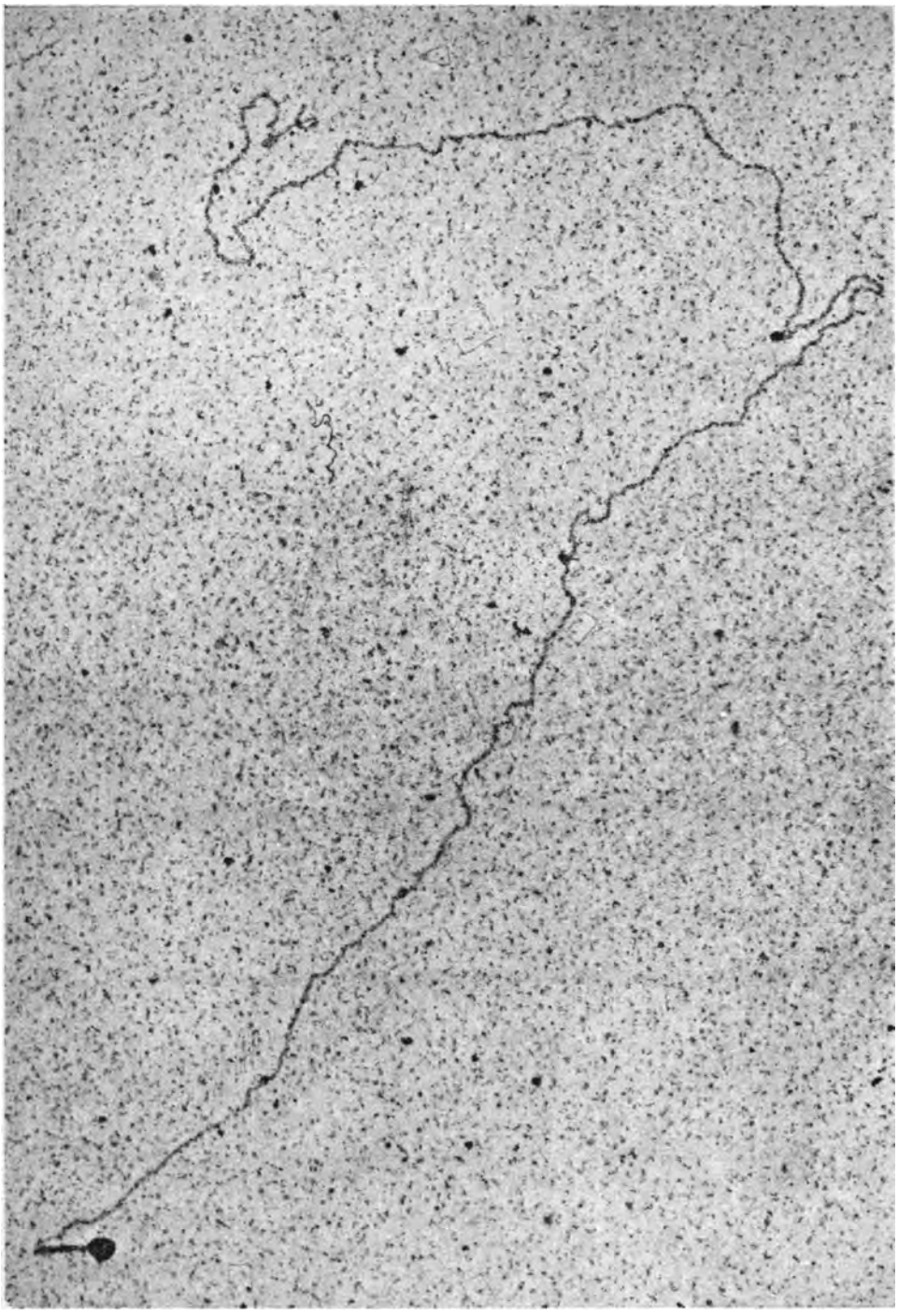

FIG. 1. Incompletely released DNA molecule from bacteriophage lambda-nin5. Measured length is 10.5 μ. × 56,000.

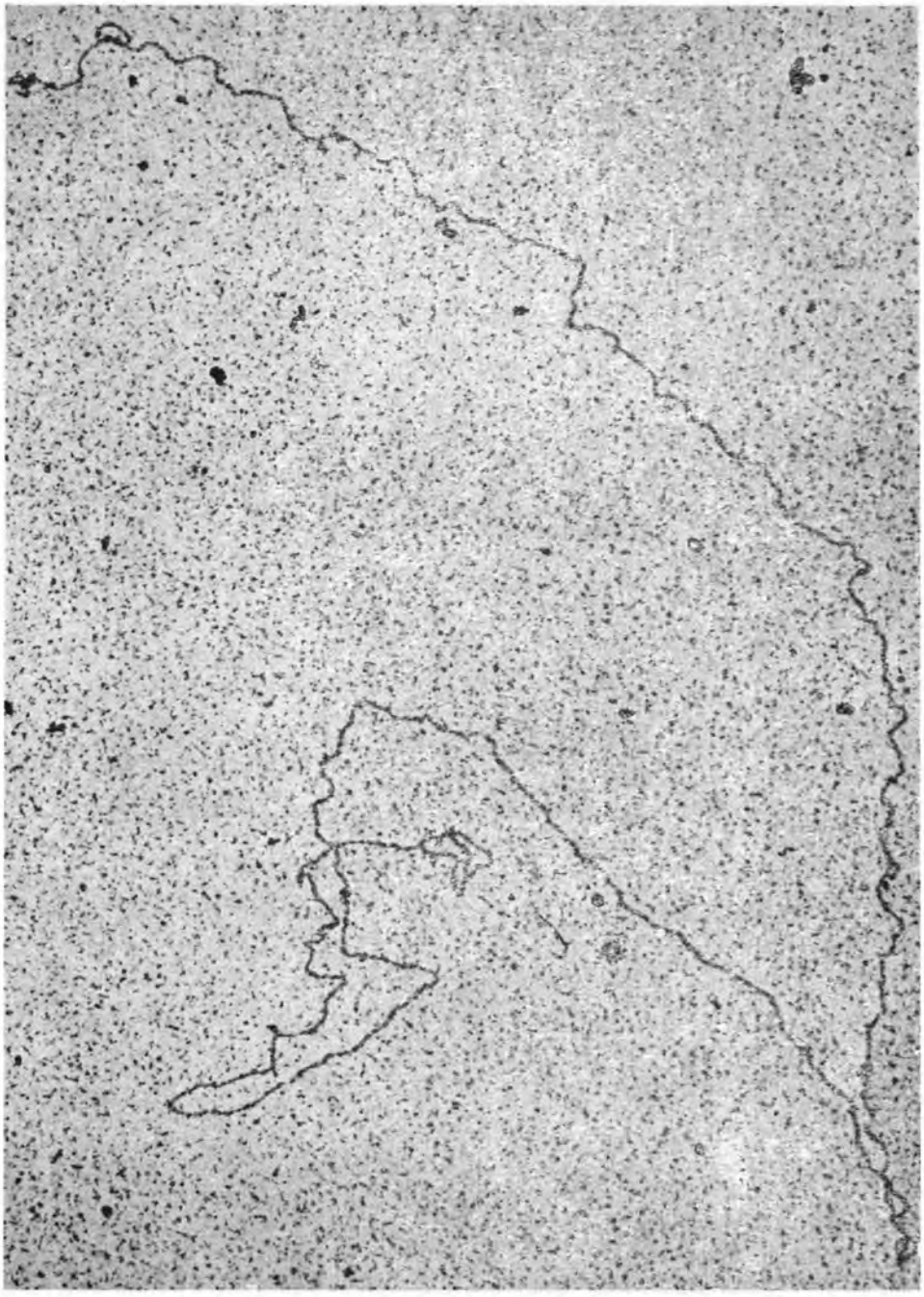

FIG. 2. Completely released linear DNA molecule from bacteriophage lambda-nin5. Measured length is 14.2 μ. × 62,000.

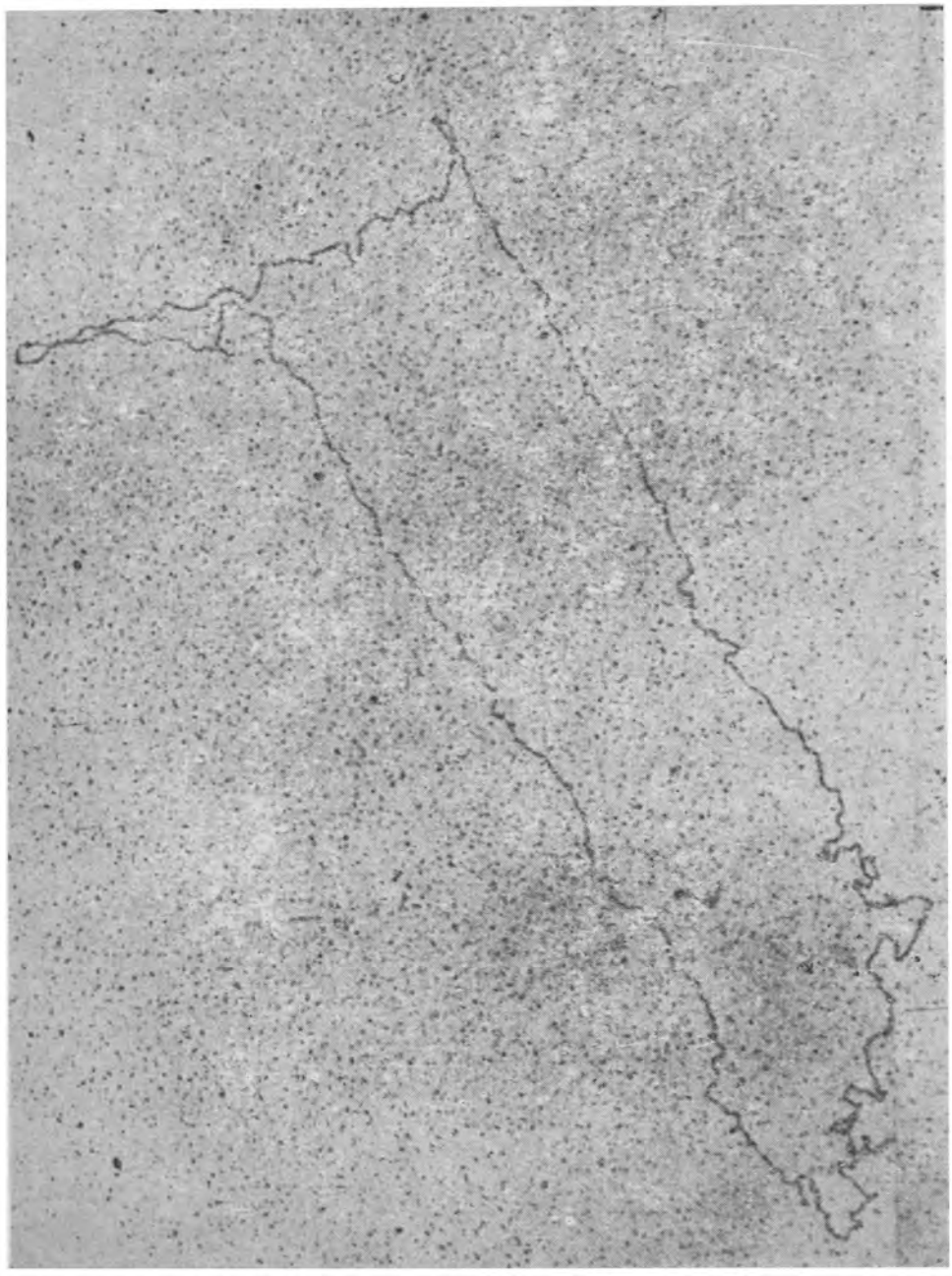

FIG. 3. Completely released endless DNA molecule from bacteriophage lambda-nin5. Measured length is 14.3 μ. × 60,000.

TABLE I

Observed length of λ DNA from wild-type phage

Author	Method of extraction	Molecular length(μ)
Ris and Chandler (1963)	Phenol	16.3
MacHattie and Thomas (1964)	Phenol	17.2
Caro (1965)	Osmotic shock	17.4
Kaiser and Inman (1965)	Alkaline digestion	15.0
Davis and Hyman (1971)	Alkaline digestion	16.0

The observed length of λ wild type DNA varied from 15–17.4μ. If lambda wild type DNA length is assumed to be 16.0μ (Davis and Hyman 1971), the observed length of the DNA from the lambda-nin5 as determined in the present work is shorter than the lambda wild-type DNA by 8.8%.

The previous estimates of the length of lambda phage DNA were done mostly with DNA released by phenol and alkalies. In contrast to these methods, the osmotic shock technique had the advantage in that the mechanical handling and pretreatment of DNA with chemicals were minimised.

Earlier length measurements were based on shadowed molecules. In shadowed specimens, the final DNA thickness is of the order of 200–250 Å due to the deposit of metal. In this procedure the very sharp kinks and bends of the molecule are usually obscured. The molecules were stained in the present study. The thickness of the stained molecules was about 100 Å. The chance of the small bends and kinks being obscured as less. Thus the estimate of DNA length based on stained preparations is more reliable.

ACKNOWLEDGEMENTS

This work was supported by the financial assistance from the University Grants Commission, New Delhi. It is a pleasure to record our indebtedness to Prof N. N. Das Gupta, for his guidance and interest; and to Dr S. Palchaudhury, for gift of the phage samples, accessories and for many helpful suggestions. Thanks are also due to Professor A. Guha, for his constructive criticism, and to Shri A. Ghosh, for helpful discussions.

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