COVALENTLY CLOSED MINICIRCULAR DNA IN MICROCOCCUS LYSODEIKTICUS

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During the search for an intracellular form of N1 phage DNA in infected M. lysodeikticus cells described in the preceding communication (Lee, Davidson, and Scaletti, 1968), we have discovered the existence of small closed circular DNA molecules (which we call minicircles) in uninfected as well as infected cells. We estimate about one minicircle per bacterium. The minicircles have a uniform contour length of 0.445 (±0.024)μ, corresponding to a molecular weight of 0.88×10^6. Very recently, Cozzarelli, Kelly, and Kornberg (1968) have found minute circular DNA molecules with a contour length of 0.96 μ in E. coli strain 15. The biological significance of these minicircles is not known.

Experimental. Some of the experimental details are described in the preceding paper (Lee, Davidson, and Scaletti, 1968). Neopeptone medium, which contains 1% Difco Neopeptone, 0.5% NaCl, 0.5% glucose, 0.002 M $MgSO_4$, is a modification of the low phosphate medium used by Kellenberger, Zichichi, and Weigle (1961). For general purposes, ML 1 bacteria were grown in 500 ml of ML broth at 32° C to $A_{600} = 0.4$ (~ 3×10^8 cells/ml). For P^{32} labeling, bacteria were grown in 500 ml of Neopeptone medium with 2-3 millicuries of carrier free P^{32} phosphoric acid. The DNA specific activities so obtained are about 4×10^4 cpm/μg M. lyso DNA and 5×10^5 cpm/μg N1 DNA. The cells were pelleted, resuspended, and gently lysed as

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described in the preceding paper. Bacterial debris and high molecular weight pieces of bacterial DNA were sedimented at 35,000 rpm for 1 hour in an SW 50L rotor. Cesium chloride and ethidium bromide were added directly to the supernatant solution. Buoyant density centrifugation in CsCl, with or without ethidium bromide, was done at 40,000 rpm for 64 hours or longer.

Before banding the P^{32} DNA, a broad background due to low molecular weight P^{32}-labeled components was partially removed by hydroxy-apatite fractionation. The lysed supernatant, after pelleting the bacterial debris, was dialyzed against 0.1 M phosphate buffer (pH 6.8). DNA was absorbed by passage through about 1 gram of hydroxy apatite on a column. Elutable counts were removed with 0.1 M phosphate washes, and the DNA eluted in a volume of 6 ml by washing with 0.3 M phosphate solution.

Because of the small amount of minicircular DNA, electron microscopy was used for detection and assay. Generally speaking, the techniques described by Davis and Davidson (1968) were used. A grid was scanned as thoroughly as possible at 2300 X (on the 35-mm film) and the minicircles were photographed at 9500 X. Contour lengths (about 20 cm for a minicircle) were measured on tracings made on Nikon shadowgraph at 50 X. A diffraction grating with 21,600 lines/cm was used in each experiment to calibrate the electron microscope.

The purity of the bacterial strains, ML 1 and ML 53-20, was in part checked by plating with phage N1. ML 1, which is sensitive to N1, gave clear plaques. No resistant bacterial colonies were present. There were no plaques at all with ML 53-20 bacteria, a uv induced mutant of ML 1, which give an abortive infection with N1 (Naylor and Burgi, 1956).

Results and Discussion. The minicircles were first observed in the fractions just below the main DNA band in a CsCl, ethidium bromide centrifugation during the search for intracellular closed circular N1 DNA described in the preceding paper. They were then observed in the same

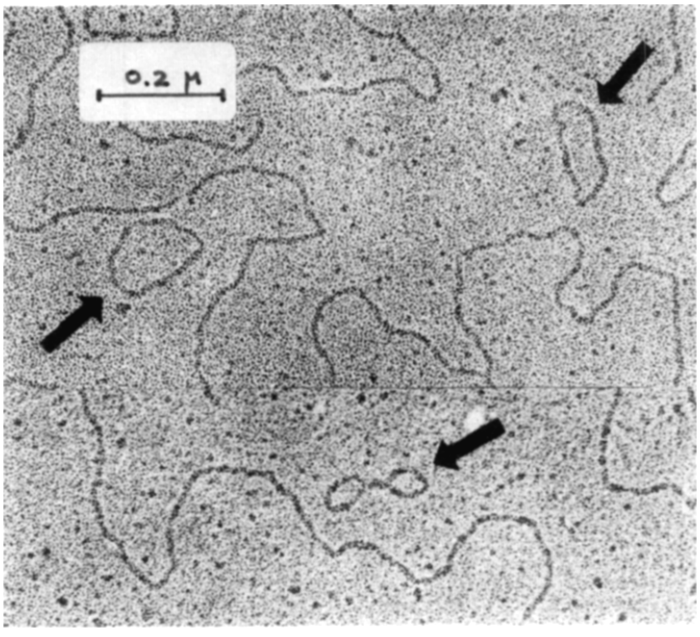

Fig. 1. Some examples of minicircles (arrows). This sample was taken from the DNA peak in Fig. 3. The specimen was stained with uranyl salt and shadowed with Pt/Pd during rotation.

fractions of DNA preparations of uninfected bacteria. They were not present in a sufficiently high concentration to be visible as a fluorescent band in the centrifuge tube. Several typical molecules are shown in Fig 1.

A histogram of the contour length distribution for measurements on 63 molecules is shown in Fig. 2. The weight average contour length is 0.445 (± 0.024)μ. By comparison with the contour length of N1 DNA which has a molecular weight of 33×10^6 (Wetmur, Davidson, and Scaletti, 1966), the molecular weight of the minicircles is calculated as $0.88(\pm 0.05) \times 10^6$.

The buoyant density of the minicircular DNA was measured by banding a P^{32} labeled M. lyso lysate in CsCl in the absence of ethidium bromide,

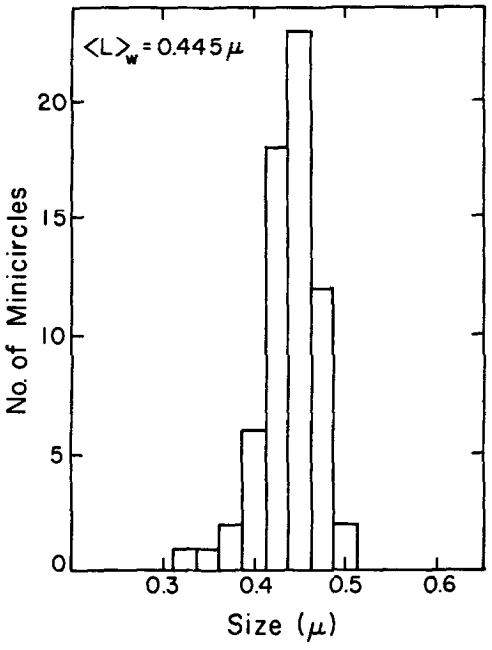

Fig. 2. The contour length distribution of minicircles (total 63 molecules). The weight average contour length is 0.445 (±0.024)μ which corresponds to the molecular weight of 0.88 (±0.05) × 10^6.

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and assaying aliquots of the same fractions for minicircles by electron microscopy. The result (Fig. 3) is that minicircular DNA has the same density as the rest of the bacterial DNA to less than the density difference between fractions (0.005 g/cc).

In the electron microscope, most of the minicircles seen had an open structure, but a few had one or two tertiary turns visible.

An approximate estimation indicated that there is about one minicircle per bacterium. The number of minicircles in a preparation was estimated from the number (usually 15-20) seen in a single square of an electron micro scope grid by assuming that the efficiency of attachment to a cytochrome film is the same for the minicircles as for N1 DNA which was mounted under identical conditions from a solution with a known DNA concentration. Cozzarelli, Kelly, and Kornberg (1968) report an average of 15 minicircles

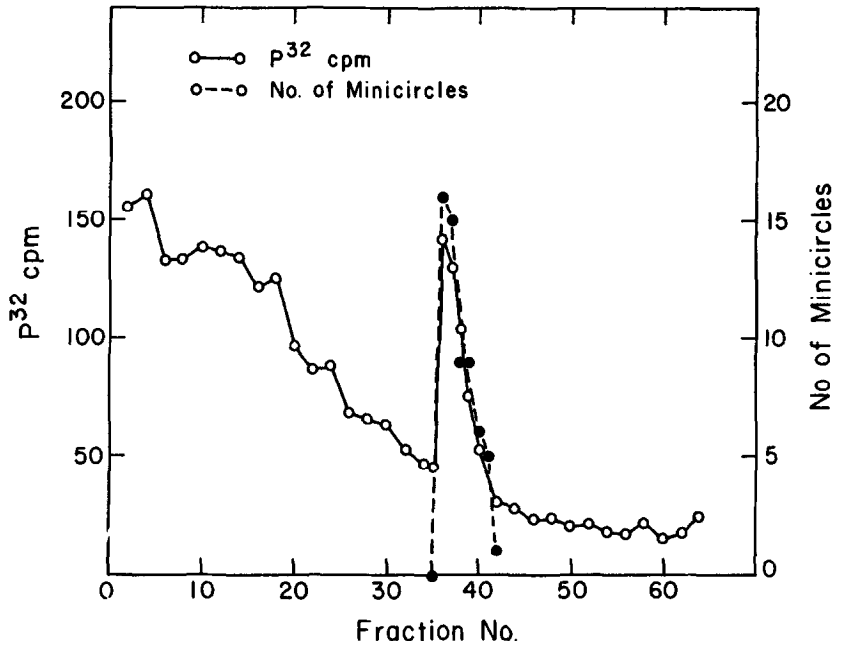

Fig. 3. The identity of the buoyant densities between minicircles and M. lysodeikticus DNA. P^{32} counts of total DNA (o ——— o) and the counts of minicircles in a square of an electron microscope grid (● - - - ●) are plotted vs. fraction number.

per cell in E. coli 15. Minicircles were found during exponential growth and in stationary cultures of ML 1. They are also found in ML53-20.

The biological significance of these minicircles, which can code for only 400 amino acids, is unknown. It should be recalled that Radloff, Bauer, and Vinograd (1967) observed circular DNA with lengths ranging down to 0.2 μ in HeLa cells, but these minute circles were not a homogeneous fraction of uniform length.

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REFERENCES

Cozzarelli, N. R., Kelly, R. B., and Kornberg, A. (1968). Proc. Natl. Acad Sci., Wash. in press.
Davis, R. W., and Davidson, N. (1968). Proc. Natl. Acad. Sci., Wash. 60, 243 (1968).
Kellenberger, G., Zichichi, M., and Weigle, J. (1961). Proc. Natl. Acad. Sci., Wash. 47, 869.
Lee, C. S., Davidson, N., and Scaletti, J. V. (1968). Biochem. Biophys. Res. Comm. preceding article.
Naylor, H. B., and Burgi, E. (1956). Virology, 2, 577.
Radloff, R., Bauer, W., and Vinograd, J. (1967). Proc. Natl. Acad. Sci., Wash. 57, 1514.
Wetmur, J. G., Davidson, N., and Scaletti, J. V. (1966). Biochem. Biophys. Res. Comm. 25, 684.