# COVALENTLY CLOSED CIRCULAR DNA FROM *MICROCOCCUS LYSODEIKTICUS* CELLS INFECTED WITH PHAGE N1

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The DNA of the *Micrococcus lysodeikticus* bacteriophage, N1, contains cohesive ends so that it is possible to convert the linear molecule to a circular form *in vitro* by annealing, and to convert the circular molecule back to the linear form by heating and quenching (Wetmur, Davidson, and Scaletti, 1966). The DNA of coliphage λ and the DNA of certain other temperate coliphages also contain cohesive ends. When the linear λ DNA molecules are injected into a bacterium upon infection, they are converted to a covalently closed twisted circular form (Young and Sinsheimer, 1964; Bode and Kaiser, 1965; Ogawa and Tomizawa, 1967; Bode and MacHattie, 1968). For λ it is also known that additional covalently closed twisted circles are made after infection (Young and Sinsheimer, 1967). Covalently closed twisted circles also occur as an intracellular form of the DNA of the *Salmonella* phage, P22. This DNA in its mature form is linear, terminally redundant, circularly permuted, but without cohesive ends (Rhoades and Thomas, 1968). In the present communication we report that covalently closed twisted circles of N1 DNA occur as an intracellular form after N1 infection also.

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Experimental. A strain of M. lysodeikticus, ML 1, and its bacteriophage, N1, were used. ML broth contains 1.5% Bactotryptone (Difco), 0.5% yeast extract (Difco), 0.5% glucose, and 0.5% NaCl (pH 7.4 before autoclaving) (Scaletti and Naylor, 1959). TE buffer is 0.01 M Tris OH, 0.01 M $Na_2H_2$ EDTA, pH 7.0. SDS solution contains 5% sodium dodecyl sulfate in TE buffer.

A 160-ml bacterial culture was grown in ML broth at 32° with vigorous aeration. One generation time is about 40 min. At $A_{600}$ = 0.6($4 \times 10^8$ cells/ml), $10^{12}$ pfu of N1 phage were added, giving a multiplicity of infection of about 16. Aliquots (40 ml) were withdrawn at 15, 30, 60, and 90 min after infection. The bacterial cells were pelleted at 7,000 rpm for 15 min in a SW 25.1 rotor, resuspended in 5 ml of TE buffer, and incubated with 2 mg of lysozyme for 15 min at 37°. After addition of 0.5 ml of SDS solution, and 0.2 ml of 2 mg/ml preincubated pronase solution, the solution was maintained at 37° for 1 hr. Cell lysis occurs and the solution becomes clear and very viscous. Bacterial debris and high molecular weight DNA are pelleted by centrifugation in a type 40 Spinco rotor at 29,000 rpm for 15 min (Kiger, Young, and Sinsheimer, 1968).

The procedure of Radloff, Bauer, and Vinograd (1967) for the separation of closed circular DNA from linear or nicked circular DNA by buoyant banding in CsCl with added ethidium bromide was followed. The CsCl and ethidium bromide were added directly to the above solution. Centrifugation of the four samples was done at 42,000 rpm for 24 hours in a SW 50 Spinco rotor. No fluorescent band due to closed circular DNA below the main band could be detected by visual examination under uv light after centrifugation (Fig. 1a). Therefore the regions below the main band in the four tubes were pooled and recentrifuged. A closed circular band was then evident (Fig. 1b). Basic protein film samples for electron microscopy were prepared from the several DNA bands by spreading a DNA-cytochrome c solution in 0.5 M salt (CsCl or a mixture of $NH_4Ac$ and CsCl)

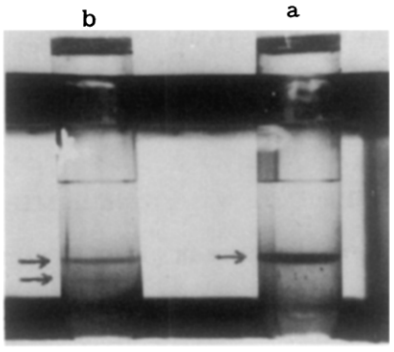


Fig. 1. Photographs under uv light of the fluorescent DNA bands (arrows) after CsCl, ethidium bromide centrifugation. (a) First centrifugation; (b) after pooling the appropriate region below the main band in (a) and recentrifuging. The lower fluorescent band contains the covalently closed circles.

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onto 0.25 M $NH_4Ac$. The grids were uranyl stained and/or rotary shadowed with Pt-Pd, followed, sometimes, by shadowing in one direction (Wetmur, Davidson, and Scaletti, 1967; Davis and Davidson, 1968).

Results and Discussion. Fig. 1b shows that there was sufficient closed circular DNA in the pooled, recentrifuged sample to give a visible band in the expected position. Linear or open circular N1 DNA and M. lyso DNA have buoyant densities in CsCl (without added ethidium bromide) of 1.718 and 1.726 and form a single band in the present CsCl-ethidium bromide procedure. Electron micrographs from the lower band showed twisted circular structures of the expected size for N1 DNA, as shown in Figs. 2a, 2d, and 2e. The lighter band contained open circular N1 DNA (Figs. 2b and 2c) and linear DNA.

N1 phages give an abortive infection in the mutant bacterium ML 53-20, derived from ML 1 (Naylor and Burgi, 1956). Excess DNA is synthesized and the cells lyse, but no phage are formed. A faint band of closed

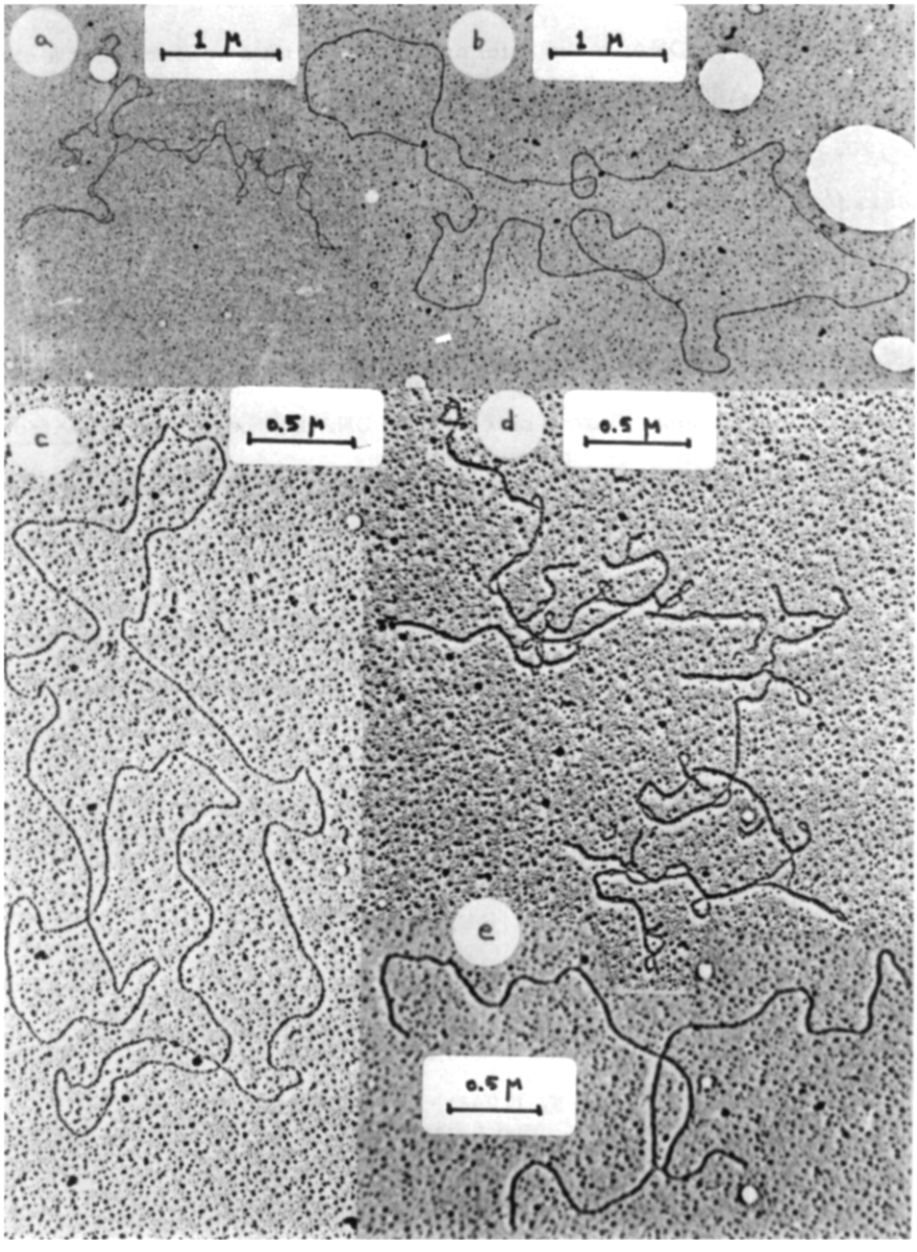


Fig. 2. Electron micrographs of twisted circles (a, d, e) and open circles (b, c) of N1 DNA from the heavy and light bands respectively. (a) and (b), stained only; (c), (d), and (e), stained and shadowed.

circular DNA was obtained after premature lysis in such an infection too.

The results thus show that a twisted closed circular form of N1 DNA is formed intracellularly after infection. It is not known whether this is newly synthesized DNA or the infecting DNA. It thus appears that a closing enzyme (ligase) exists in M. lyso cells. This is in accord with the high uv resistance of the bacterium and possibly with the findings of Elder and Beers (1965) dealing with the repair capacity of M. lyso extracts for uv damaged transforming DNA.

The result confirms the expectation that the formation of intracellular covalently closed supertwisted circles after phage infection is a rather common phenomenon. Closed circular N1 DNA has been made in vitro (Wang, Baumgarten, and Olivera, 1967). Thus, physical studies on in vivo and in vitro closed circular molecules with a high GC content (70%) are now possible.

Further studies on the time course of formation of the closed circular DNA after infection and of its significance in the infection process are now being made.

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