SHORT COMMUNICATION

MULTIPLE FOLDING OF THE SPREAD CHROMOSOMES OF *ANACYSTIS NIDULANS*

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Chromosomes of blue-green algae have been studied by electron microscopy in *Anabaena cylindrica* (Hopwood and Glauert 1960), *Anacystis nidulans*, *Calothrix paristina*, *Gloeocapsa alpicola*, *Fischerella muscicola* (Ris and Singh 1961), *Symploca muscarum* (Pankratz and Bowen 1963), *Anacystis* sp. (Leak 1965) and *Anacystis variabilis* (Leak 1967). According to these papers the chromosomes were reported to be about 2-6 nm in width. In addition to these papers biochemical analyses of the chromosome of *Anacystis nidulans* revealed that the quantity of DNA per cell was $3.0-3.6 \times 10^{-14}$ g (Echlin 1966). Sedimentation coefficient (S) of the chromosomal DNA of *Anacystis nidulans* was 30-32 (Kung *et al.* 1972). They reported that by this sedimented feature the DNA double helix of *Anacystis nidulans* might be linear. In the present investigation the chromosome of *Anacystis nidulans* (*Synechococcus*, Berkeley strain No. 6301) obtained from Berkeley Culture Collection, University of California, was studied by the electron microscopic protein monolayer technique of Kleinschmidt and Zahn (1959).

Anacystis nidulans was grown at 30°C for 3 days in the medium C of Kratz and Meyers (1955). The culture was continuously gassed with air containing 1% CO₂ and illuminated with fluorescent lights at 2,400 lux. The exponentially growing 8×10^7 cells were incubated at 30°C for 30 h by a reciprocating shaker in 20 ml of 0.6 M sucrose solution containing 10.0 mg lysozyme (Sigma) adjusted with 0.05 M Tris-HCl buffer solution to pH 8.0. By the lysozyme treatment spheroplasts were obtained. The prepared spheroplasts were centrifuged at $9,000 \times g$ in the cold for 10 min, and washed three times with 0.6 M sucrose. Then, 2×10^4 spheroplasts were suspended finally into 0.6 M sucrose containing 0.01% cytochrome c (Sigma, Type III) and spread on distilled water at 25°C. The spread spheroplasts were picked up on 150-mesh copper grids coated with carbon-formval, dehydrated in ethanol series, immersed in isoamyl acetate and air dried. The air dried spheroplasts were stained with 2% uranyl acetate for 5 min, and rotary-shadowed with 8:2 platinum-palladium mixture at an angle of about 7°, and stabilized by applying a thin coat of carbon. Then, they were observed with a Hitachi HS-9 electron microscope at magnification of $3,000 \times$ to $10,000 \times$ at 75 kV.

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Fig. 1. Electron micrograph of the chromosome of *Anacystis nidulans* spread on distilled water by whole mount method. The bar is 1,000 nm.

In spread spheroplasts on distilled water, high electron dense membranes and low electron dense membranes were observed. Chromosomes appeared spreading around these membranes. The chromosomes were observed to be attached to these membranes by their some regions. Most of the membranes were observed to be flat and some to be fibrous which were low electron dense (Fig. 1).

The spread chromosomes were observed to be composed of the continuous and long chromosome fibers with almost uniform width. It was a notable feature that the chromosome fibers were folded into coils forming a structure of several turns of the chromosome fibers (Figs. 1 and 2). Most of the coils were observed to be folded into three to eight turns, while the smallest one was only one turn and the largest one observed was more than 12. About half of the coils were observed to be gathered together and made up the larger coil. In those cases the former coils were named as the primary

Fig. 2. Electron micrograph of multiple-folded coils in the chromosome of *Anacystis nidulans*. The bar is 200 nm.

coil and the latter as the secondary coil. The secondary coils were found to be composed of two or three primary coils which were encircled with the chromosome fiber one to four times. Some of the primary coils and the secondary coils, furthermore, gathered together making up the tertiary coil which was composed of one to about five primary coils and one to about five secondary coils which were surrounded with the chromosome fiber one to four times. The diameter of the primary coil varied from about 80 to 600 nm, that of the secondary coil from about 300 to 800 nm, and that of the tertiary coil varied from about 400 to 1,800 nm. Thus, the chromosomes spread on distilled water showed the coil structure which was successively folded into a multiple-folded coil (Fig. 2).

The multiple-folded coil was observed in 21 out of the 30 cells examined. Rest the nine cells showed only primary coils. The number of multiple-folded coils per cell varied from two to eight.

Delius and Worcel (1973, 1974) reported that the chromosomes of *Escherichia coli* spread on a hypophase containing 20% formamide and 0.01 M Tris formed a coil structure which was similar to the present primary coil. According to their observation,

however, the secondary coil and the tertiary coil were not observed. In order to make sure the present observation, the chromosomes of *E. coli* (K12 W1895) were spread by the same method as one applied to *Anacystis nidulans*. As compared with *E. coli*, more primary coils were observed in the chromosomes of *Anacystis nidulans*. While some chromosomes without terminal end were observed, some well spread chromosomes with terminal ends that varied from one to six in number per cell were observed. In these well spread chromosomes supertwisted regions were observed additionally. The supertwisted regions occurred localizing in the chromosome fibers without coils (Fig. 1). In contrast to the presume of Kung *et al.* (1972) that the DNA fiber of *Anacystis nidulans* might be linear, in the present investigation the occurrence of supertwisted region may indicate that the chromosome of *Anacystis nidulans* is composed of partially or fully circular DNA, as presumed in the chromosomes of *E. coli* through the occurrence of supertwists (Worcel and Burgi 1972; Pettijohn and Hecht 1973).

Judging from the structure of continuously uniform width and the same width as the DNA fibers of *E. coli* (Delius and Worcel 1973, 1974), the chromosome fibers observed in the present *Anacystis nidulans* are presumed to be the DNA double helix itself.

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