THE FINE STRUCTURE OF THE CELL BOUNDARY OF THE BLUE-GREEN ALGA *ANACYSTIS MONTANA*

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In the course of an investigation into the fine structure of the blue-green alga *Anacystis montana*,[1] it was found that the outermost layer of the cell boundary is convoluted into a series of blebs or protrusions (Figs. 1 to 3). The cell boundary of blue-green algae has previously been described by Ris and Singh (1) as consisting of three layers: a bipartite inner or plasma membrane, an inner investment, and an outer membrane which in some species was found to be slightly wrinkled.

[1] The organism was sent to me by Dr. L. Provasoli of the Haskins Laboratory, New York, as a species of *Synechococcus*. It was subsequently identified as *Anacystis montana fa. minor* (*Wille*) Drouet and Daily (5), (6) by Dr. F. Drouet of the Academy of Natural Sciences, Philadelphia.

The mucilaginous sheath is a separate entity and lies outside the outer membrane of the cell boundary. This present study agrees with findings of Ris and Singh with the exception that the outer part of the cell boundary is more highly convoluted than previous reports have shown.

The material was fixed in 6 per cent glutaraldehyde in a phosphate-sucrose buffer, pH 6.5, for 48 hours at 4°C and washed several times with cold phosphate-sucrose buffer. Postfixation was carried out in either 1 per cent osmium tetroxide in veronal-acetate buffer + sodium chloride, pH 6.1, (2) for 16 hours at 4°C, or in 0.6 per cent potassium permanganate in veronal-acetate buffer, pH 7.4, (3) for 1 hour at 0°C. The convolutions were less pronounced in material fixed in permanganate alone. Specimens were dehydrated through an ethanol series, embedded in Epon 812, and sections were stained in 10 per cent aqueous phosphotungstic acid for 5 minutes, washed in distilled water, and examined in an RCA EMU 3F electron microscope.

The convolutions or blebs appear to be fairly regularly spaced around the cell periphery and are between 40 and 80 mμ high. The electron opaque outer membrane is between 75 and 80 A thick and forms the outer boundary of the convolutions. The electron transparent inner investment is of varying thickness and forms the inner part of the convolutions.

The electron opaque inner membrane is 100 to 150 A thick and does not seem to play any part in the structure of the convolutions. Remnants of the sheath material may be seen outside the outer membrane (Figs. 1 to 3). It is unlikely that the convolutions represent a fixation artefact as they have appeared regularly in material grown under different conditions and fixed by a variety of methods.

It is possible that the convolutions of the outer membrane may represent either a structural link between it and the narrow sheath material found in *Anacystis* or be connected with the synthesis or formation of the cell envelope. Hopwood and Glauert (4), working with *Anabaena cylindrica*, found long fibrils extending from the outermost layer of the cell boundary into the sheath material, which in *Anabaena* is considerably thicker than in *Anacystis*.

This study represents part of an investigation into the fine structure of the blue-green algae initiated at the Section of Cytology and Genetics, School of Medicine, University of Pennsylvania, Philadelphia. The work was supported in part by research grants from the National Institutes of Health, United States Public Health Service, PHS No. CA-03010 and PHS No. AI-02203.

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FIGURES 1 TO 3

The three electron micrographs show the convolutions in the outermost layers of the cell boundary of *Anacystis montana*. The material in all three micrographs was fixed in buffered glutaraldehyde followed by postfixation in buffered osmium tetroxide. Sections were stained for 5 minutes in 10 per cent phosphotungstic acid. Cell boundary, *cb*; outer membrane, *om*; inner investment, *ii*; inner or plasma membrane, *im*; mucilaginous sheath material, *ms*. The scale on all three micrographs represents 0.1 micron. Magnifications: Fig. 1, × 40,000; Fig. 2, × 105,000; Fig. 3, × 120,000.