Fine Structure of Swarmers of *Cladophora* and *Chaetomorpha*

III. Wall Synthesis and Development

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Received May 2, 1972

**Summary.** Naked swarmers of *Cladophora* have been collected and wall synthesis and development have been followed using the techniques of freeze-etching and sectioning. Swarmers frozen after 9 hours liberation have lost their flagella, developed the characteristic fibrous layer and show the initial stages of wall production. Both the first formed (randomly oriented) and the later (more ordered) microfibrils appear to have a distinct granular texture. Occasionally linear arrays of granules up to 4 μm long may be seen. After 5 days settling a thick wall composed of almost transversely oriented microfibrils is present and a rhizoid is pushed out. Also characteristic of this stage is the central localisation of cell components and peripheral vacuolar distribution. Longitudinally oriented microtubules also reappear at this stage having been absent during earlier wall formation.

A possible relationship between the cortical microtubules of the motile swarmer and the development of the fibrous layer is suggested.

**Introduction**

In the previous two papers in this series (Robinson and Preston, 1971a; Robinson, 1972) we initiated an investigation of the total fine structure of a “naked” plant cell, namely the swarmers of the two filamentous green algae *Cladophora* and *Chaetomorpha*, with a view to determining how the cellulose microfibrils of the wall are synthesized. Good freeze-etch replicas revealing cytoplasm-wall relationships were not however obtained, although with *Chaetomorpha* the replicas showed granules to be present near the plasmalemma surface carrying “tails” which might be considered microfibril initials.

Since this material is available only for short periods each year two other green algae were studied. The first of these, *Glaucocystis nostochinearum* (Robinson and Preston, 1971b, 1971c), was not very satisfactory either but the alga *Oocystis apiculata* proved to be an admirable substitute for the swarmers (Robinson and Preston, 1972). The freeze-etch replicas obtained using *Oocystis* as source material, have gone a long way toward substantiating the involvement of plasmalemma granules.
in cellulose microfibril biosynthesis. However, although the disposition of plasmalemma granules in *Oocystis* corresponds to that of the microfibrils of the wall, attachment of granules to microfibrils was never seen. The freezing procedure used with *Oocystis*, namely the use of cardboard discs in place of copper and the avoidance of cryoprotective agents, appeared to be a distinct advance and it was thought desirable to apply the same technique to *Cladophora* swarmers.

**Materials and Method**

Specimens of *Cladophora rupestris* were collected and swarmers liberated as described in Robinson and Preston (1971a). Swarmers were collected over a period of 9 hours and placed in a beaker in a refrigerator at 3°C. A portion of this material (in which some of the swarmers had already settled) was then centrifuged gently and the cells frozen immediately on cardboard discs without any cryoprotective treatment. The remainder of the material was transferred to a small aquarium tank at the base of which was a tight-fitting glass plate previously roughened by sand-grinding. The tank, with a loose fitting cover was placed in a culture room at 8°C for 5 days at a light intensity of 800 lux and a photoperiod of 9 h. After this settling period the cells were washed off the glass plate by a gentle stream of water. The cells were then centrifuged, some were frozen as above and the rest were removed for sectioning. The freeze-etch replicates were prepared on a machine constructed in this department (now available as the N.G.N. 600 Freeze-etcher). Etching (for a period of $1^{1/2}$ min), replication and cleaning were carried out as described elsewhere (Robinson and Preston, 1971b). For sectioning the material was fixed (2 hrs at 3°C in 4% glutaraldehyde in 0.075 M cacodylate buffer, pH 7.0; containing 0.25 M sucrose), washed in buffer (sucrose concentration decreasing) and then postfixed (5 hrs at 3°C) in 2% OsO$_4$ in 0.075 M cacodylate buffer (pH 7.0). After dehydration through an alcohol series and propyleneoxide the cells were embedded in Spurr Low Viscosity Resin, (Polyscience Inc., Penn) (Spurr, 1969). Sections were cut on a Porter-Blum MT-2 microtome using a diamond knife (Rondikin Corpn., Delaware) and stained with uranyl acetate and lead citrate.

Electron micrographs were taken in a Philips EM200 at 60 or 80 kV. Light micrographs were taken in a Zeiss Ultraphot II.

Fig. 1. Part of a convex fractured swarmer of *Cladophora*. Note the cross-fractured fibrous layer and granular layer (arrow) and the striations over the surface of the plasmalemma. ×18000

Fig. 2. High power of part of Fig. 1 revealing the striations to be in fact grooves. Notice the 20 nm granules. ×48800

Fig. 3. A concave surface fracture of a *Cladophora* swarmer during wall synthesis. The fracture plane is thought to have passed through the granular layer near the fibrous layer rather than at the level of the plasmalemma. Note the fibrillar network. ×10800

Fig. 4. High power of part of Fig. 3. Note the granularity of the "microfibrils" (20 nm diameter) and the matrix containing granules at the top of the micrograph. ×40800