Spatial relationship between microtubules and plasma-membrane rosettes during the deposition of primary wall microfibrils in *Closterium* sp.

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**Abstract.** The mechanism by which cortical microtubules (MTs) control the orientation of cellulose microfibril deposition in elongating plant cells was investigated in cells of the green alga, *Closterium* sp., preserved by ultrarapid freezing. Cellulose microfibrils deposited during formation of the primary cell wall are oriented circumferentially, parallel to cortical MTs underlying the plasma membrane. Some of the microfibrils curve away from the prevailing circumferential orientation but then return to it. Freeze-fracture electron microscopy shows short rows of particle rosettes on the P-face of the plasma membrane, also oriented perpendicular to the long axis of the cell. Previous studies of algae and higher plants have provided evidence that such rosettes are involved in the deposition of cellulose microfibrils. The position of the rosettes relative to the underlying MTs was visualized by deep etching, which caused much of the plasma membrane to collapse. Membrane supported by the MTs and small areas around the rosettes resisted collapse. The rosettes were found between, or adjacent to, MTs, not directly on top of them. Rows of rosettes were often at a slight angle to the MTs. Some evidence of a periodic structure connecting the MTs to the plasma membrane was apparent in freeze-etch micrographs. We propose that rosettes are not actively or directly guided by MTs, but instead move within membrane channels delimited by cortical MTs attached to the plasma membrane, propelled by forces derived from the polymerization and crystallization of cellulose microfibrils. More widely spaced MTs presumably allow greater lateral freedom of movement of the rosette complexes and result in a more meandering pattern of deposition of the cellulose fibrils in the cell wall.

**Key words:** Chlorophyta - *Closterium* - Cell wall (primary) - Cellulose deposition - Microfibril - Microtubule - Plasma-membrane rosette.

**Introduction**

A role for microtubules (MTs) in the orientation of cellulose microfibrils in plant cell walls was first postulated by Ledbetter and Porter (1963) based on the observation that these structures are often aligned parallel to one another during microfibril deposition. How MTs bring about this orientation is still poorly understood. Theoretical analysis of the problem indicates that a minimum of three different components must be involved: cellulose-polymerase enzymes localized in the plasma membrane, cortical MTs, and structures by which the MTs interact with the plasma membrane to guide the polymerases (Heath 1974; Heath and Seagull 1982; Lloyd 1982; Staehelin and Giddings 1982; Roberts et al. 1985).

There is mounting evidence that cellulose-synthase complexes are membrane-bound and are associated with distinct configurations of particles in freeze-fractured plasma membranes (reviewed by Brown 1985). These complexes have been visualized as particle rosettes on the P-face (fracture faces identified according to Branton et al. 1975) of the plasma membrane at the ends of elongating microfibrils in vascular plants (e.g., Mueller and Brown 1980; Herth 1983) and some green algae (Giddings et al. 1980; Herth 1983). E-face globules complementary to the rosettes have been reported in some systems but not in others (see e.g. Schneider and Herth 1985). In many other algae, the purported cellulose-synthase complexes take the form of linear bands of E-face or P-face particles (e.g., *Oocystis*: Brown and Montezinos 1976; *Glaucocystis*: Willison and Brown 1978; *Pelvetia":

**Abbreviations:** E-face = exoplasmic fracture face; MT = microtubule; P-face = protoplasmic fracture-face
Fig. 1. Cortical MTs in a growing semicell of Closterium sp. The primary cell wall (CW) and a waxy outer layer (OL) are shown. High-voltage electron micrograph of 0.25 μm thick section. × 100000; bar = 0.1 μm

Peng and Jaffe (1976). Each rosette or linear band of particles is believed to contain a number of cellulose-synthase molecules, each of which polymerizes a cellulose glucan chain that becomes laterally associated with other chains to form elementary fibrils and microfibrils (Giddings et al. 1980). Since the postulated cellulose-synthase complexes always appear associated with the tips of growing microfibrils, it has been postulated that as the fibrils are formed, the complexes move forward in the membrane, thereby wrapping the fibril around the cell. Recent quantitative studies of rosette densities and rates of cell-wall fibril deposition further indicate that a given particle rosette may spin out approx. 1 μm of cellulose per 1 min, and that the lifespan of one rosette could be on the order of 10 min (Schnepf et al. 1985; Schneider and Herth 1986). However, direct biochemical evidence in support of this theory is still lacking.

To investigate the mechanism by which MTs control microfibril orientation, we have examined the spatial relationship of particle rosettes and cortical MTs during semicell formation in a species of Closterium, a single-celled green alga. A spindle-shaped desmid with two identical semicells at interphase, Closterium undergoes cytokinesis at the isthmus, where the two semicells are joined. Each semicell then regenerates a new, complementary semicell. During growth of the primary cell wall of the new semicell, cortical MTs and microfibrils are both oriented circumferentially (Pickett-Heaps and Fowke 1970; Hogetsu and Shibaoka 1978a; Hogetsu and Oshima 1985). Hogetsu and Shibaoka (1978b) demonstrated that colchicine depolymerizes the cortical MTs and causes a random pattern of microfibril deposition and the formation of a spherical rather than a spindle-shaped semicell. Microtubules have been postulated to play a role in confining cellulose synthesis to the new semicell (Hogetsu and Takeuchi 1982). Particle rosettes in the plasma membrane are associated with the formation of cellulose microfibrils in both primary and secondary cell walls in Closterium (Staehelin and Giddings 1982; Hogetsu 1983). In this paper, we present morphological evidence in support of the hypothesis that membrane-associated cortical MTs define membrane channels within which the particle rosettes are constrained to travel during cellulose synthesis, thereby controlling the orientation of the deposited fibrils.

Material and methods

The Closterium sp. used in this study was isolated locally by Dr. J.D. Pickett-Heaps. It was grown in the medium of Pocock (1960) at 16°C under a 15 h light-9 h dark cycle. About half of the cells divided within a 2-h period each day.

High-voltage electron microscopy. Cells were fixed at room temperature in 1% glutaraldehyde, 0.2% tannic acid in 50 mM K-phosphate buffer (pH 6.8). After 10 s of exposure to fixative, aqueous OsO₄ in K-phosphate buffer was added to a final concentration of 0.1%. At 15-min intervals, the solution was replaced with fresh glutaraldehyde-tannic acid fixative. After 1.5 h, the cells were rinsed in K-phosphate, post-fixed in 1% OsO₄ in buffer for 1 h, dehydrated in a graded ethanol series and two changes of dimethoxypropane, and embedded in Spurr's resin (Spurr 1969). Sections 0.25 μm thick were stained.