MICROTUBULES AND FIBRILS IN THE CYTOPLASM OF *COLEUS* CELLS UNDERGOING SECONDARY WALL DEPOSITION

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Much attention is currently being devoted to the nature of the mechanisms in the cytoplasm which control and orient the deposition of polysaccharide microfibrils on the inner surface of the plant cell wall (1–4). We have approached the question of cytoplasmic participation in wall deposition by studying the fine structure of cells undergoing rapid secondary wall formation. Our study has demonstrated the presence of two different cytoplasmic components which appear to be associated with the rapidly developing secondary wall: (1) microtubular structures aligned in the cytoplasm adjacent to the secondary wall; and (2) clusters of fibrillar elements within cisternae of the endoplasmic reticulum. We believe these two components are different from one another, and, in order to distinguish clearly between them, will hereafter refer to them as "tubules" and "fibrils," respectively.

The plant material used in the investigation consisted of stem segments of *Coleus blumei* Benth. which had been excised, wounded, cultured on agar, and supplied with indoleacetic acid at the apical end (5, 6). Parenchyma cells interior to a vascular bundle severed by the wounding were induced by this treatment to redifferentiate into tracheary elements, and, in the process, to lay down numerous bands or reticulations of secondary wall. These redifferentiating cells were used in the investigation of fine structure. They were excised after 4 days of culture, fixed in glutaraldehyde followed by osmium tetroxide (7), stained in a solution of uranyl acetate (8), and embedded in an Epon-Araldite resin mixture. Sections were cut with a diamond knife and mounted on bare 400-mesh grids. In some cases sections were poststained with lead hydroxide (9).

Secondary wall deposition in the redifferentiating cells is preceded by the appearance of thick bands of cytoplasm in the thin peripheral cytoplasmic layer of the large, vacuolate cells (10). These cytoplasmic bands are rich in elements of the endoplasmic reticulum, Golgi bodies, vesicles, and mitochondria (6). Deposition of secondary wall material results within 4 to 5 days in the appearance of scalariform, anastomosing bands consisting of massive depositions of cellulose, lignin, and other wall components.

The tubules were localized beneath the plasmalemma in the cytoplasm next to the developing bands of secondary wall (Fig. 1). In sections normal to the wall bands, the tubules were observed in cross-section arranged around the bands (Fig. 1, inset). Similar structures were not positively identified adjacent to the primary wall, an observation which may be related to the fact that the primary wall was no longer growing. Tubules associated with a particular wall band ran parallel to its surface and were usually aligned roughly parallel to one another in the direction taken by the band. They were sectioned longitudinally and could be traced for some distance through the cytoplasm when the region of transition between wall and cytoplasm lay within the plane of section (Figs. 1 and 5). The tubules were generally straight, although some exhibited a slight curvature or small angular changes in direction. They measured from 220 to 280 A in diameter, and consisted of a dense wall 55 to 70 A thick and a light interior 110 to 140 A in diameter. Their lengths were indeterminate, although some of the tubules were traced through the cytoplasm for at least 2 μ. They were not observed in material fixed in permanganate.

The nature and function of the tubules are unknown. Microtubular structures have been widely reported in animals and among the protista (reviewed by Slautterback, 11), and have been demonstrated by Manton in the spermatozoids of a moss (12) and of a fern (13). Quite recently, cytoplasmic tubules resembling those reported here have been found in higher plants by Ledbetter and Porter (14) in close association with the growing primary walls of root tips. An association of similar structures with the secondary wall has not been previously reported, so far as we are aware. We conjecture that the tubules may be concerned in some way with the development of the wall, *e.g.*, by determining the place of deposition and the orientation of the

wall microfibrils. Our results with cells fixed in permanganate, which appears to delineate the cellulose microfibrils by staining the associated lignin, indicate that the wall microfibrils exhibit the same general orientation as the cytoplasmic tubules.

The second distinctive component in the cells undergoing rapid secondary wall development consisted of groups of what we have interpreted to be fibrils in various stages of development lying within the cisternae of the granular reticulum (Figs. 2 to 4). In Fig. 2, sections of fibrils in an apparently rather early stage of development are seen within a cisterna near a band of secondary wall. While we sometimes observed profiles of cisternae containing clusters of fibrils lying in the cytoplasm without any obvious orientation with respect to a wall band, in other cases they lay near and parallel to the bands (Fig. 3). We also observed cisternae containing fibrils and aligned parallel to one another and at right angles to the long axis of wall bands (Fig. 5). The fibrils within the cisternae contained a dense, thick cortex or wall and a lighter, narrow core. The total diameter of the fibril was 120 to 150 A, or little more than half that of the tubules. Some of the fibril aggregates were traceable for 1 to 2 μ. The fibrils were sinuous in form, suggesting flexibility in contrast to the rigidity suggested by the straightness or slight angularities of the tubules. Like the tubules, the fibrils were not observed in permanganate-fixed material.

It seems likely that the fibrils contain a proteinaceous component, in view of their electron opacity and their preservation when doubly fixed with glutaraldehyde and osmium tetroxide but not when fixed in permanganate. A considerable polysaccharide component, particularly in the less dense core, is, of course, not excluded. Our reasons for suggesting that the fibrils may be incorporated in whole or in part into the wall include: their abundance in cells in which the formation of secondary wall substances must dominate the synthetic processes; their considerable length, suggestive of the long microfibrils of the cell wall; their proximity to the wall bands and their frequent orientation with respect to them; and finally, their localization within the endoplasmic reticulum, a phenomenon which may be analogous to the well known accumulation of products in this system prior to their secretion in animal cells. Whether these fibrils do indeed represent developmental stages of the secondary wall microfibrils, or contain polysaccharide cores which are in some way released and then aggregated into microfibrils on the inner surface of the wall, are questions which remain to be investigated.

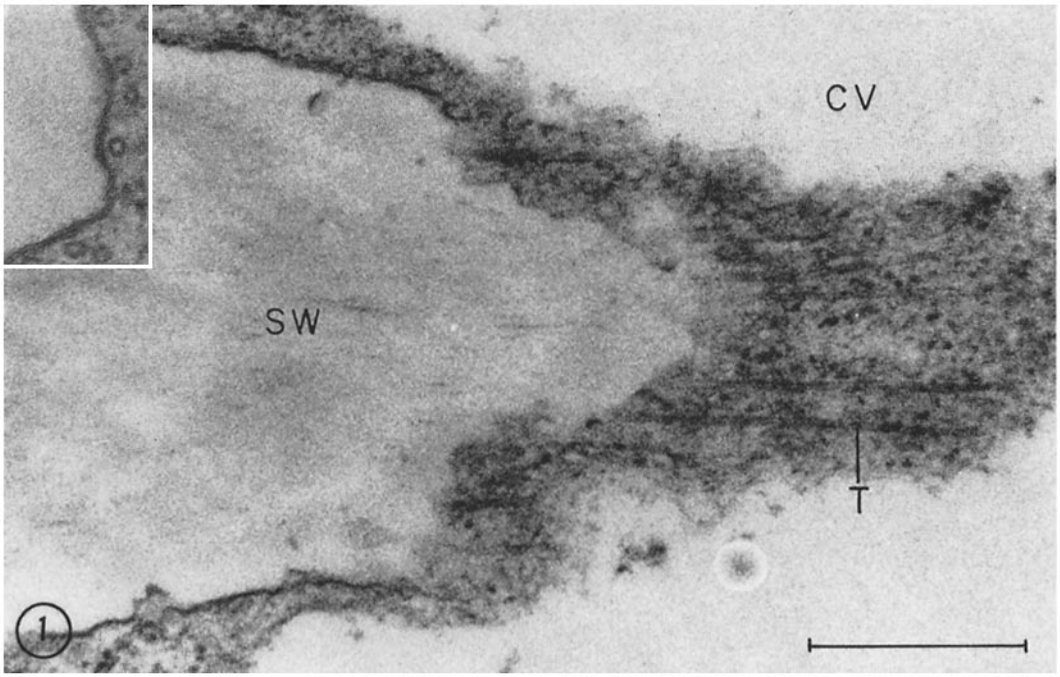
Explanation of Figures

The length of the solid line on each micrograph represents 0.5 micron.

T, tubule	*ER*, endoplasmic reticulum
SW, secondary wall	*PW*, primary wall
CV, central vacuole	*M*, mitochondrion
F, fibrils	*P*, polyribosome

FIGURE 1 Oblique section through a developing secondary wall band in a *Coleus* stem parenchyma cell redifferentiating into a tracheary element. The region of transition between wall and cytoplasm is included in the plane of section, and reveals tubules running nearly parallel to one another in the cytoplasm in the direction of the wall band and parallel to its surface. Inset (upper left) shows cross-sections of tubules lying in the cytoplasm and separated from the secondary wall on the left by the plasmalemma. × 59,000. Inset, × 100,000.

FIGURE 2 Portion of a parenchyma cell redifferentiating into a tracheary element, showing a cisterna of the granular reticulum lying near a developing band of secondary wall cut transversely. Transverse, oblique, and longitudinal sections of structures interpreted as fibrils in an early stage of development are evident within the cisterna. Section poststained with lead hydroxide. × 42,000.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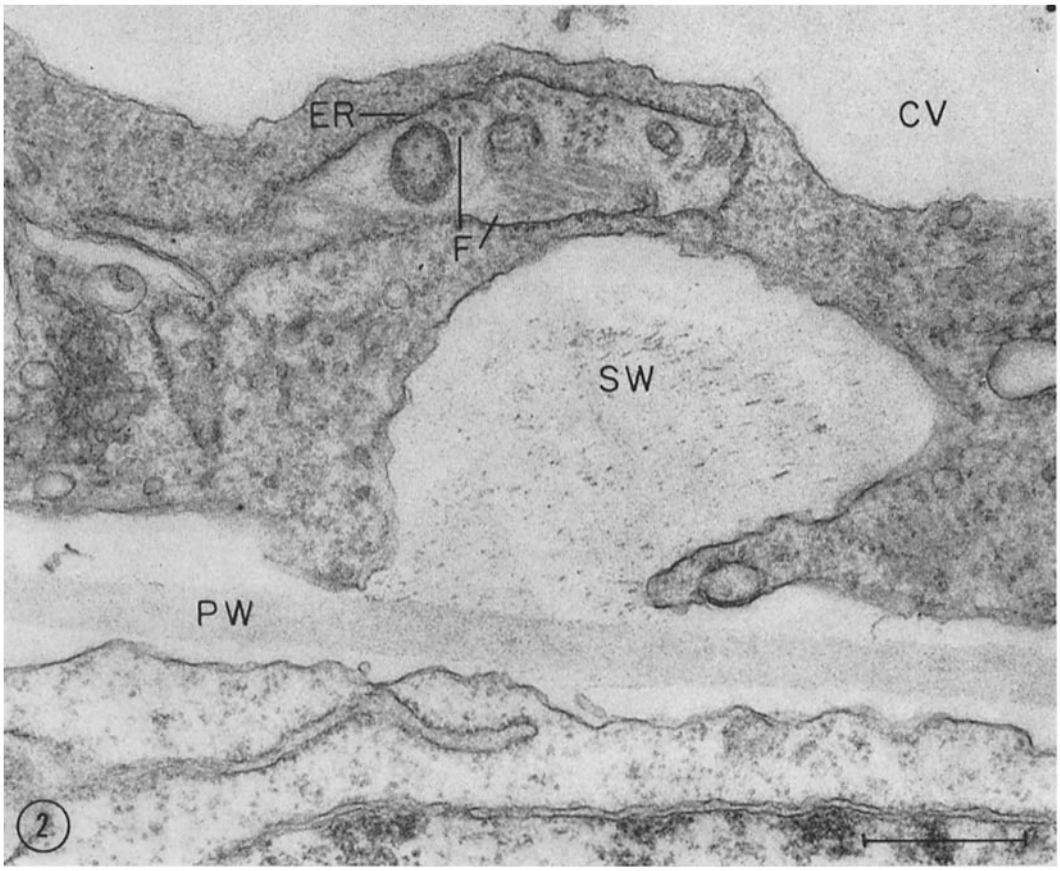
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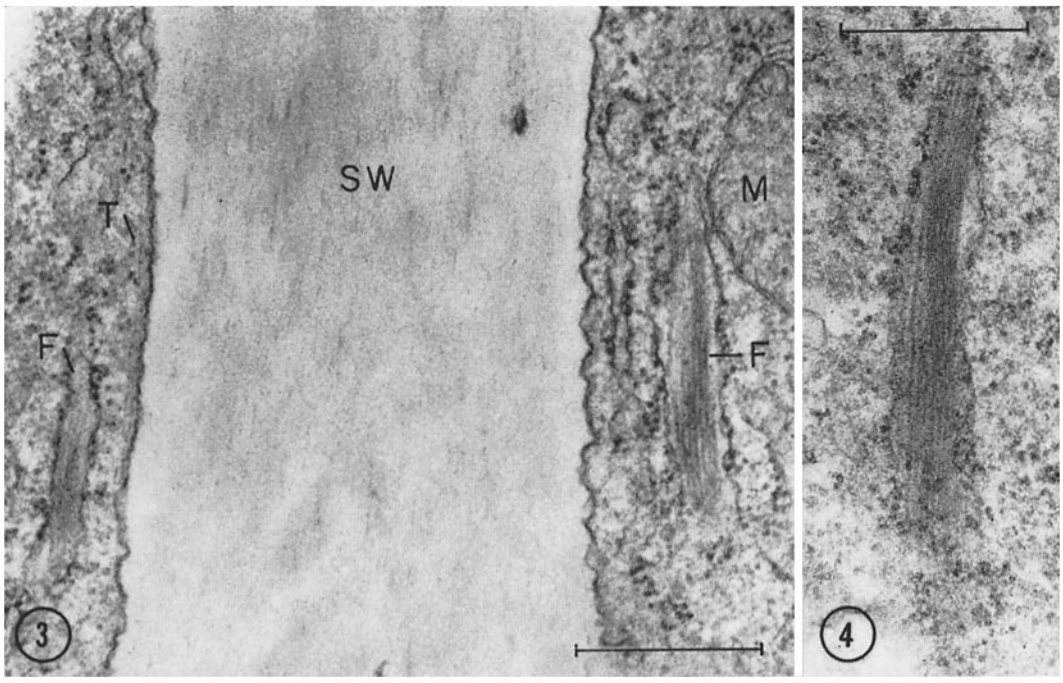
FIGURE 3 Portion of a redifferentiating cell showing the cytoplasm in the vicinity of a tangentially sectioned developing band of secondary wall. Two cisternae of the granular reticulum, each containing a group of fibrils, lie in the cytoplasm parallel to the long axis of the wall band. × 49,000.

FIGURE 4 Profile of a cisterna of the granular reticulum containing a number of fibrils. × 50,000.

FIGURE 5 Oblique section through a developing band of secondary wall showing the cytoplasm in the vicinity of the band. Three bundles of fibrils within cisternae of the granular reticulum lie parallel to one another and at right angles to the long axis of the wall. The cisternae are evident at the sides of the wall, but not in the central region because they curve out of the plane of section in conforming to the bulge of the wall into the cytoplasm. Several tubules run through the cytoplasm between the cisternae and the wall and parallel to the long axis of the latter. Short striations in the wall indicate the orientation of the wall microfibrils. × 60,000.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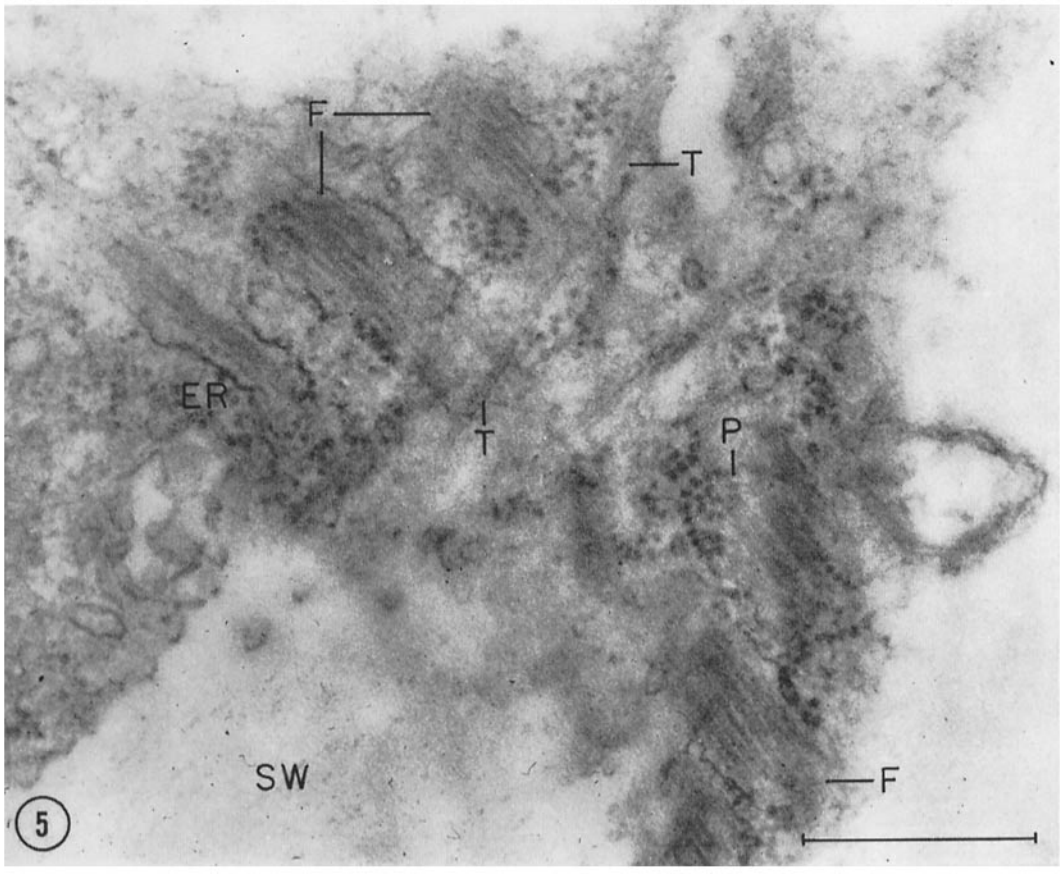
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