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WORLD-WIDE RESEARCH

Fine Structure of the Cytoplasm in Relation to the Plant Cell Wall

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Our present knowledge of the disposition of microtubules in the cytoplasm of plant cells is summarized. The reports to date indicate that the orientation of the microtubules of the cell cortex mirrors that of the cellulose microfibrils in the adjacent wall, and it is suggested that the tubules in some way dictate the orientation of the microfibrils. An examination of the fine structure of the microtubule reveals that its wall is made of elements which are aligned in 13 files parallel to the long axis of the tubule. This structure is compared to that reported for the tubular component of sperm tails from animals.

Many characteristics of plant products—such as fruit texture, fiber strength in cotton, structural properties of wood—are related to chemical make-up and architecture of the plant cell wall. The means by which the protoplast of the cell may determine its wall morphology is of interest to agronomists as well as students of cell differentiation.

Over a century ago Crüger (*3*) described a curious relationship of the cytoplasm to the deposition of patterned wall thickenings. He examined the behavior of certain cells which develop spiral thickenings and saw, prior to any detectable secondary wall growth, bars of streaming cytoplasm under which the wall thickenings subsequently appear. In a contemporary study of the physical make-up of the cell wall, Nägeli used polarized light to demonstrate its crystalline nature, a fact which eventually led to the discovery that the long cellulose molecules of the wall are grouped together into microfibrils.

During some 80 years which followed Crüger's work, similar observations were made by others; and the hypothesis gained acceptance that secondary thickenings and the orientation of cellulose microfibrils in the wall are determined by streaming patterns of the cytoplasm. The discovery of some exceptions led van Iterson in 1942 (*6*) to the postulate that anisotropy of some component of the ectoplasm (or the cortex of the protoplast) is in some way responsible for the orientation of cellulose micelles.

A recent study (*8*) of the fine structure of the cortex of plant cells actively engaged in wall synthesis revealed an array of units which renders the cortex anisotropic. The elements are tubular in form, of minute dimensions, and abundant in the interphase cell cortex and in the mitotic spindle. These microtubules from plant cell cortices have been characterized in some structural detail (*9*). The report which follows summarizes our knowledge of the relationship of these cytoplasmic elements to orientation of the cellulose microfibrils in the cell wall and the structural similarity of the plant microtubules to similar elements found in certain animal cells.

Materials and Methods

There is reason to believe that the basic mechanisms of cell wall formation are essentially similar in various species of higher plants and even in diverse cell types. This permits us a wide choice of materials for investigation.

For this study we examined the cell cortices in root tips of *Phleum pratense* L. and *Juniperus chinensis* L., and the nectaries of *Euphorbia Milii*, Ch. de Moulins. These tissues were fixed in glutaraldehyde, a preservative which has proved

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superior in the preservation of fine structure, and postfixed in osmium tetroxide (*7*, *14*). After the tissues were dehydrated and plastic-embedded, thin sections of the cells were cut with a diamond knife, stained with heavy metal salts, and observed in an electron microscope.

Tilting of the section to aid in interpretation of fine structure of the microtubule was accomplished with a stereo device of the microscope.

Results

The microtubules which have been observed in the cell cortex have a diameter of about 230 to 270 A., and are of undetermined length. Some have been traced for several microns in a single section, and it was not evident that the ends were contained in the section. From cross and longitudinal sections they appear to be tubular in form, being made of a wall some 70 A. thick enclosing a lumen about 100 A. in diameter.

In the interphase cell the microtubules are most abundant in a cortical zone about 1000 A. thick just subjacent to the plasma membrane (Figure 1). Examination of the side walls in a barrel-shaped meristematic cell of root tips shows that the microtubules are oriented circumferentially around the cell, much as hoops around a barrel. Thus, in cross sections of root tips the tubules adjacent to side walls of the cell lie within the plane of sectioning (Figure 1) while in longitudinal sections of the root tip the microtubules may be cut transversely (Figure 2), displaying their tubular nature as circles of high density. At the end walls of these meristematic root tip cells the microtubules are in a more or less random array (*9*). Along both the side walls and end walls of these cells, the microtubules mirror the orientation of the cellulose microfibrils in the adjacent wall.

The tubules have not been observed in contact with one another or with ribosomes. There is a zone of low density around each microtubule (Figure 2), from which some structures seem to be excluded. However, contacts are found between the tubules and the plasma membrane, the tonoplast (Figure 4), and the "pectin vesicles" of the phragmoplast (Figure 3). During formation of the phragmoplast a fine fibrous material is associated with the microtubules (Figure 3).

When the cell enters mitosis the microtubules are no longer seen in its cortex. The "spindle fibers" which develop in the mitotic apparatus are made of tubular elements having a morphology similar to the cortical microtubules though possibly smaller in diameter (*8*).

In cross-sectional view it is occasionally possible to see that the wall of the microtubule is composed of small units. This is particularly evident in certain cells of *Juniperus* and *Euphorbia*. The contents of these cells are extraordinarily dense, imparting a natural "negative staining" to some structures, especially the cortical microtubules. In our first studies of the tubules seen in this way Markham's rotation technique was used to show that the wall of the microtubule is probably composed of 13 subunits (*9*). Subsequent images at improved resolution (Figure 5) permit direct confirmation of these results.

In the electron microscope it is possible to tilt the specimen to obtain views of an object from various angles. Figure 6 shows microtubules from two aspects which differ by 10°. It is evident that this amount of tilt from the optimum viewing angle prohibits us from seeing the details of fine structure in the wall of the tubule.

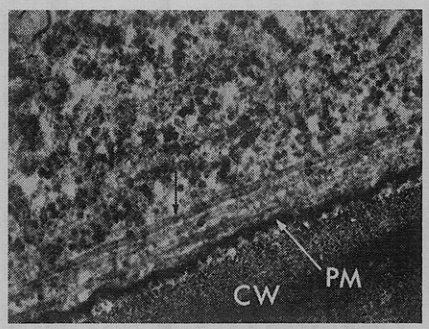

Figure 1. Electron micrograph of cortical cytoplasm from *Juniperus* root tip in transverse section with microtubules (arrow), plasma membrane, *PM*, adjacent to cell wall, CW

×47,000

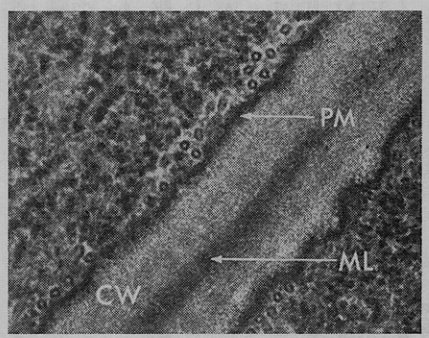

Figure 2. Microtubules (dense circles) and plasma membrane, *PM*, adjacent to cell wall, CW, with middle lamella, *ML*, from longitudinal section of *Juniperus* root tip

×51,000

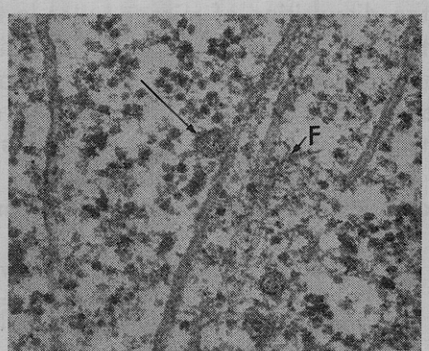

Figure 3. Portion of phragmoplast from *Juniperus* with pectin vesicle (arrow), fibrous material, *F*, along microtubules

×65,000

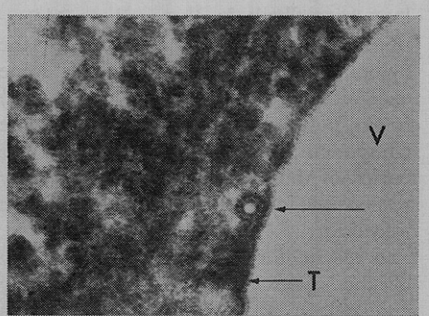

Figure 4. Microtubule (arrow) attached to tonoplast, *T*, of vacuole, *V*, in nectary of *Euphorbia*

×123,000

Discussion

The orientation of these tubular elements mirrors that of the adjacent cellulose microfibrils not only in the meristematic cells examined here, but also in the secondary thickenings of cells examined by Hepler and Newcomb (*5*). In these cells cytoplasmic streaming is known to occur in bands over the developing secondary thickenings (*15*) in a manner similar to those reported by Crüger (*3*). It may be that the orientation of cellulose microfibrils is generally correlated with cytoplasmic movement, at least in the outermost cortical layer of the cytoplasm. Exceptions have been noted in *Nitella* and the stamen hairs of *Tradescantia*, where the direction of observable cytoplasmic streaming does not coincide with the orientation of cellulose microfibrils. It is well known that in *Nitella* the outer cortical layer of cytoplasm, in which the chloroplasts are buried, behaves differently from the inner portions of the protoplast, where streaming is so evident. Movements of the cytoplasm near the growing wall may be minute and transversely oriented as are the long axes of the microfibrils. In *Tradescentia*, wall growth by deposition of transverse

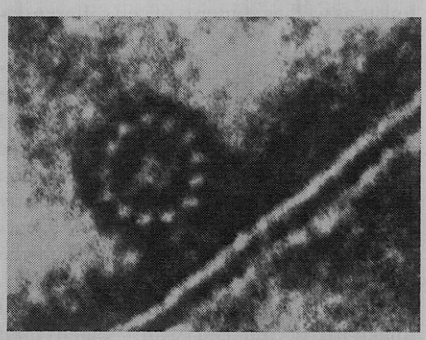

Figure 5. Microtubule from Juniperus in "natural negative staining," showing 13 files of elements in wall

×650,000

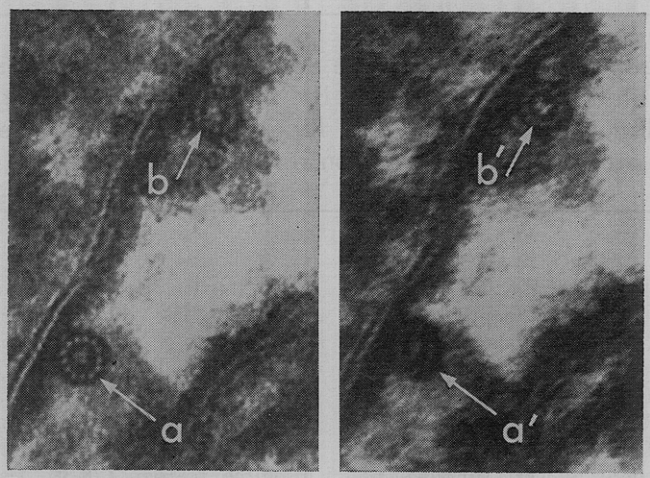

Figure 6. Microtubules from *Juniperus* seen from aspects which differ by 10°

Clear image, *a*, blurred when tilted, *a′*; blurred image, *b*, becomes clear when tilted, *b′*. ×220,000

cellulose microfibrils may be complete prior to the observed longitudinally oriented cyclosis. The mechanism by which cytoplasmic streaming or the presence of microtubules might orient the microfibrils of cellulose is difficult to fathom, especially since the locus of cellulose synthesis is unknown. In this connection, the intimate association of the microtubules with the membranes is of interest. Information may be transmitted in some way by the plasma membrane to the growing cell wall.

In their general morphology and details of fine structure the microtubules in cells of higher plants are similar to units found widely distributed in plants and animals (*2, 4, 5, 8–11, 13, 16*). It is evident from the results reported here that the wall of the tubule as viewed in transverse section is composed of 13 units and that the axis of the tubule must coincide with that of the electron beam of the microscope to within a very few degrees if the subunits are to be resolved. Such structure has been resolved in a section with an estimated thickness of 1500 A. This suggests that the wall of the tubular element is composed of an array of filaments parallel to the axis of the tubule and that these filaments must follow lines parallel to the axis to within a few angstroms for at least 1500 A. This concept compares favorably with the negatively stained images of whole tubules from the 9 + 2 complex of sperm tails (*1, 12*). The role of these tubular elements found widely distributed among plant and animal cells is probably one of providing a cytoskeletal structure which is associated with cytoplasmic movement and cell asymmetries (*2, 13*).

There is ample reason to believe that the cytoplasm controls the specific patterns of cellulose microfibrillar orientation found in the cell walls of higher plants. It is reasonable then to expect to find in the cytoplasm some anisotropic mechanism for this control. The system of cortical microtubules described here fulfills this expectation far better than any other structure of the cell cortex known to us.

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WORLD-WIDE RESEARCH

Modification of Wheat Protein for Preparation of Milk-Like Products

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MANY UNDERDEVELOPED COUNTRIES have an insufficient amount of protein food, including low-cost protein-rich beverages suitable for small children. The present work was undertaken to explore the possibility of converting wheat protein into a dry material that would reconstitute with water to give a milk-like product. The product should be bland in flavor, milk-like in appearance (white), and have a protein solid ratio similar to milk. Lysine addition to such a material would be desirable to provide a somewhat better amino acid balance than is found in wheat. Such a material might be employed in combination with other high quality protein sources such as nonfat dry milk or soybean protein. Processing costs may limit the general applicability of methods of modifying wheat protein.

Problems in producing a milk-like

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