THE ULTRASTRUCTURE OF THE DEVELOPING
SOROPHORE OF
MARSILEA VESTITA

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ABSTRACT

In the young sporocarp of Marsilea vestita, the sorophore tissue consists of vacuolate parenchymatous cells. The vacuoles contain electron-dense bodies which disappear as the cells enlarge. During the differentiation of the sorophore cells, there are three phases of polysaccharide accumulation. During the first phase, speroidal carbohydrate bodies accumulate in vesicles and vacuoles of the peripheral cytoplasm. Globular carbohydrate bodies which are interconnected by strands of polysaccharide appear in the central vacuole during the second phase. The third phase begins with the appearance of strands of polysaccharide radiating out into the central vacuole from the tonoplast. As the sorophore cells mature, the central vacuoles become filled with this fibrous polysaccharide. The cytoplasm ultimately degenerates leaving behind dispersed globular carbohydrate and fibrous polysaccharide. When the mature sporocarp is scarified and placed in water, the hygroscopic polysaccharide in the sorophore cells expands and stretches the cell walls. As a result, the sorophore is transformed into a long column many times its original length.

THE SOROPHORE of the fern Marsilea has fascinated students of plant morphology for many years. This tissue expands into a long mucilaginous column when the sporocarp is scarified and hydrated. Bilderback (1978) has demonstrated that the developing sorophore cells accumulate massive amounts of hygroscopic mucilage. However, little is known about the mode of polysaccharide accumulation during the differentiation of the sorophore.

MATERIALS AND METHODS—Sporocarps of Marsilea vestita Hook. et Grev. were collected from the shores of Ninepipes Reservoir, Charlo, Montana, and fixed in 5% gluteraldehyde buffered with 0.05 M sodium cacodylate, pH 7.2, and postfixed in buffered 2% osmium. The tissue was rinsed twice in buffer, dehydrated in a graded ethanol series and embedded in the medium of Spurr (1969). Silver and silver-gold sections were stained either with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) or by the method of Thiéry (1967) for polysaccharides.

RESULTS—2–3-mm sporocarp—Early in the development of the sporocarp, the sorophore tissue consists of vacuolated cells with thin walls traversed by plasmadesmata (Fig. 1, 2). The tissue is interspersed with angular intercellular air spaces. The vacuoles contain individual or clusters of electron-dense bodies. The most prominent organelle is the nucleus with a conspicuous nucleolus and regions of condensed chromatin. The cytoplasm contains numerous polysomes as well as free ribosomes but little endoplasmic reticulum (Fig. 2). Small vacuoles, dictyosomes and lipid droplets are observed occasionally. The matrices and stromae of the mitochondria and plastids are electron dense and contain ribo-
somes. The cristae of the mitochondria are well developed; however, the internal lamellae of the plastids are organized only into long linear associations. A few plastids have small starch grains.

As the development of the sporocarp proceeds, the sporophore cells enlarge, and the cytoplasm becomes restricted to a thin peripheral layer around a large central vacuole (Fig. 3). Except for the deposition of starch in a few plastids, little change has occurred in the ultrastructural cytology of the cells.

4-mm sporocarp—Many sporophore cells have vacuoles containing fibrillar material which is heavily stained by uranyl acetate and lead citrate (Fig. 4). This material has become highly condensed in a few cells. Profiles of endoplasmic reticulum have increased, and microtubules can be observed adjacent to the cell wall.

5-mm sporocarp—The plasmalemma of the sporophore cells no longer is situated adjacent to the microfibrillar portion of the cell wall and only makes contact at localized points along the wall (Fig. 5, 7, 9). The pericytoplasmic space stains weakly for polysaccharides but on close inspection contains fine fibrillar material. Occasionally, the plasmalemma forms large invaginations around regions containing chains of spheroidal bodies of polysaccharide which appear to aggregate into a larger body (Fig. 7). In addition, individual polysaccharide bodies can be observed along the main portion of cell wall, and arrays of microtubules are oriented along the plasmalemma. The plasmalemma, as well as the membranes of the endoplasmic reticulum, dictyosomes and mitochondria stain intensely for polysaccharides but are poorly defined by osmium alone (Fig. 5–9, 12). Presumably, the intensity of the staining reaction results from glycoproteins associated with these membranes. On the other hand, the tonoplast stains poorly for polysaccharides (Fig. 5). The number of dictyosomes has increased significantly (Fig. 9). The small vesicles associated with many dictyosomes either stain lightly and uniformly for polysaccharide or contain fibrillar material (Fig. 8). However, the vesicles associated with a few dictyosomes contain small, dense aggregates of spheroidal bodies of polysaccharide (Fig. 12). There are many large vacuoles which contain substantial amounts of carbohydrate in varying degrees of condensation (Fig. 9). Some of these carbohydrate bodies resemble the vacuolar bodies observed in the cytoplasm of the sporophore tissue of the 4-mm sporocarp. Stands of polysaccharide and associated spheroidal bodies commonly are observed radiating outward from many of the condensed carbohydrate bodies (Fig. 9, 10). With increased magnification, the carbohydrate bodies consist of aggregates of spheroidal bodies of various dimensions (Fig. 11).

6-mm sporocarp—There has been a massive deposition of globular carbohydrate bodies in the large central vacuole (Fig. 13). These bodies appear to consist of aggregates of small spheroidal bodies of polysaccharide (Fig. 14). Characteristically, the carbohydrate bodies are interconnected by thin strands of polysaccharide (Fig. 13, 14). The peripheral cytoplasm still has small vacuoles containing carbohydrate bodies, and the tonoplast does not stain for polysaccharides (Fig. 13).

7-8-mm sporocarp—When stained with uranyl acetate and lead citrate, the cells of sporophore exhibit variable cytological characteristics. Certain cells have normal cytoplasm with ribosomes, small vesicles and well-defined membranes (Fig. 15, 17). Occasionally, concentric arrays of membranes are found in the central vacuole (Fig. 16). Other cells may have densely staining cytoplasm with intact membranous systems (Fig. 20). However, a few cells have degenerating electron-dense cytoplasm with disorganized or no membranous components (Fig. 15, 18). The degenerated cells may lie adjacent to normal cells in the sporophore tissue (Fig. 15).

The cell walls also have undergone considerable modification. There is evidence of both localized as well as massive deposition of new and different polysaccharides (Fig. 17, 28). Occasionally, polymerized contents of vesicles appear to be fusing with the cell wall (Fig. 18), and the

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Fig. 8–16. 8. Dictyosome with small lightly stained vesicles and a large vesicle containing fibrous material (arrow). 5-mm sporocarp. ×28,735. 9. Cytoplasm of a sporophore cell from a 5-mm sporocarp with dictyosomes (D) and vacuoles containing carbohydrate bodies (C). ×11,900. 10. Carbohydrate body (C) with strands of polysaccharide (arrows). Small spheroidal carbohydrate bodies are associated with the strands. 5-mm sporocarp. ×33,700. 11. Detail of a carbohydrate body. 5-mm sporocarp. ×99,665. 12. Dictyosome with associated vesicles containing small, spheroidal carbohydrate bodies (arrows). 5-mm sporocarp. ×54,165. 13. Sorophore cell from a 6-mm sporocarp with globular carbohydrate bodies in the central vacuole (V). ×15,125. 14. Detail of the vacuolar carbohydrate bodies interconnected by strands of polysaccharide. 6-mm sporocarp. ×91,000. 15. The cell wall (CW) between two adjacent sporophore cells from an 8-mm sporocarp. One cell on the right is normal while the other cell is degenerating. ×40,615. 16. Concentric array of membranes in the central vacuole. 8-mm sporocarp. ×130,664.
cytoplasm often is separated from the main portion of the wall by the deposition of a loose network of polysaccharide fibrils (Fig. 19).

Many sorophore cells have large vacuoles which extend from the cytoplasm into the central vacuole (Fig. 20). These vacuoles contain large, electron-dense bodies, some of which appear to be homogeneous in structure. Others are decidedly heterogeneous with many spherical electron-translucent regions (Fig. 21).

When stained for polysaccharides, the cytoplasm of the sorophore cells also exhibits variable cytological characteristics. Some cells have electron-transparent cytoplasm (Fig. 22). The chromatin of these cells is dispersed evenly within the nuclear membrane (Fig. 23). Other cells have cytoplasm which stains heavily for polysaccharides (Fig. 22, 24, 26, 29). The chromatin of these cells is condensed and uniformly stained (Fig. 24). In both types of cells, the tonoplast now stains intensely for polysaccharides (Fig. 22-24).

As the cells of the sorophore continue to mature, strands of polysaccharide begin to appear in the central vacuole which may still contain small, spherical bodies of polysaccharide as well as large, globular carbohydrate bodies (Fig. 25). These strands of polysaccharide originate at the tonoplast and consist of many individual fibrils (Fig. 25-27). The dictyosomes and associated vesicles appear to be contributing only to cell wall modification and not to the formation of the polysaccharide strands (Fig. 28). However, as the cytoplasm degenerates, vesicles may release their contents into the central vacuole (Fig. 29). During the final phase of differentiation, the number of polysaccharide strands increases, and they fill the central vacuole (Fig. 29, 30). All the strands appear to originate at the tonoplast. The peripheral cytoplasm ultimately degenerates, leaving just polysaccharide within the cell walls.

**Germinated sporocarp**—When scarified and placed in water, the sporocarp splits open and the sorophore emerges. The sorophore cells contain dense aggregates of globular carbohydrate and strands of polysaccharide (Fig. 31). The walls consist of a loose network of fibrils and are approximately 1.5–2 times thinner than the walls of maturing sorophore cells in the 7–8-mm sporocarp (Fig. 32).

**Discussion**—Early in the development of the sporocarp, the sorophore tissue consists of small, vacuolate parenchymatous cells. The vacuoles contain conspicuous electron-dense bodies. Other tissues of the sporocarp have these vacuolar bodies and have been presumed to be protein (Bilderback, 1978). As the sorophore tissue enlarges and forms a central vacuole, these inclusions decrease in size and number and finally disappear altogether. The role of these vacuolar inclusions during the differentiation of the sorophore has not been elucidated.

There are three phases of polysaccharide accumulation during the development of the sorophore cells. During the first phase, there appears to be some differentiation of dictyosomal function. Some dictyosomes are involved with cell wall modification. Other dictyosomes have associated vesicles which contain spherical bodies of carbohydrate. These vesicles apparently fuse with one another to form small vacuoles which in turn fuse with other vesicles and vacuoles to form larger vacuoles containing globular bodies of carbohydrate. There is some evidence that the carbohydrate bodies also contain protein. Some of these protein-carbohydrate bodies persist as the sorophore cells differentiate. Bilderback (1978) has observed dense bodies which stain for both carbohydrate and protein embedded in the mucilaginous matrix of mature sorophore cells.

During the second phase, large numbers of globular carbohydrate bodies accumulate in the central vacuoles of the sorophore cells. These bodies are interconnected by fine strands of polysaccharide. There is some similarity between the carbohydrate that appears in small vacuoles during the first phase and the carbohydrate now found in the central vacuole. It is possible that the carbohydrate originates in small vacuoles which then fuse with the tonoplast releasing their contents into the central vacuole.

During the first two phases of polysaccharide accumulation, the tonoplast does not stain for polysaccharide and presumably contains little or
no glycoprotein. However, with the onset of the third phase, there is a profound change in the tonoplast. The membrane can be readily stained for polysaccharides. Associated with this change in the tonoplast, long strands of the polysaccharide begin to appear in the central vacuole. At first, only a few strands consisting of a number of individual fibrils are seen radiating out from the tonoplast. Presumably, the strands are being polymerized by a small group of synthetases which are restricted to a particular region of the tonoplast. However, as maturation of the sorophore tissue continues, many sites of polymerization appear on the tonoplast, and the central vacuole becomes filled with the fibrous polysaccharide. The cytoplasm ultimately degenerates leaving behind a matrix of fibrous polysaccharide in the cells. The fibrous polysaccharide has been found to contain the two sugars, rhamnose and arabinose (Bilderback, 1978). These sugars are common constituents of mucilage from higher plants (Robinson, 1967).

The epidermal cells of Plantago ovata seeds differentiate also as mucilage cells (Hyde, 1970). The mucilage of these cells is synthesized by dictyosomes and accumulates not only in vacuoles but between the plasmalemma and the cell wall. The central vacuole disappears when the tonoplast fuses with the plasmalemma. The contents of the vacuole become continuous with the material accumulated in the pericytoplasmic space. The cytoplasm is progressively restricted to one portion of the cell and ultimately degenerates. The mucilage of these cells resembles the carbohydrate found in the sorophore cells during the first two phases of polysaccharide accumulation.

There is considerable ultrastructural evidence that the walls of sorophore cells undergo profound changes during development. The plasmalemma separates from the microfibrillar portion of the wall, and new polysaccharides are deposited in the pericytoplasmic space. In the young sporocarp the thin walls are traversed by many plasmadesmata. As differentiation of the cells proceeds, the number of plasmadesmata decrease significantly. Individual cells appear to undergo independent differentiation, and degenerating cells are commonly found adjacent to normal cells in the sorophore tissue.

The sorophore mucilage is hygroscopic and rapidly expands when the mature sporocarp is scarified and placed in water. In response to the expanding mucilage, the cell walls of the sorophore are transformed from thick, compact structures into thin, porous ones. In fact, the walls become so porous that the fibrous polysaccharide readily diffuses into the aqueous medium surrounding the expanding sorophore (Bilderback, 1978).

LITERATURE CITED


Fig. 25–32. 25. A strand of polysaccharide (arrow) radiating into the central vacuole which contains globular carbohydrate bodies (C). A plastid with dark staining grana contains a large starch grain (S). 7-mm sporocarp. ×23,330. 26. A strand of polysaccharide extending into the central vacuole from the tonoplast. The cytoplasm and cell wall (CW) stain heavily for polysaccharide. 8-mm sporocarp. ×49,045. 27. Detail of a polysaccharide strand consisting of many individual polymers of polysaccharide. 8-mm sporocarp. ×38,890. 28. Dictyosomes and associated vesicles containing polysaccharide. One vesicle has recently fused with the heterogeneous cell wall (CW). 8-mm sporocarp. ×39,400. 29. Vesicles apparently releasing spheroidal carbohydrate bodies to a central vacuole containing fibrous polysaccharide. 8-mm sporocarp. ×99,665. 30. Two adjacent sorophore cells from an 8-mm sporocarp. The central vacuole of the cell on the left is filled with fibrous polysaccharide. The cytoplasm of the cell on the right has degenerated completely leaving the fibrous polysaccharide. ×27,550. 31. Sorophore cells from a germinating sporocarp containing globular carbohydrate bodies and fibrils of polysaccharide (arrow). ×25,000. 32. Detail of the sorophore cell wall from a germinating sporocarp. ×83,660.