

Tetsuko Noguchi

The cell wall is an extracellular structure that encloses each cell in land plants, algae, and fungi. It is more rigid, thicker, and stronger than the extracellular matrix produced by animal cells. Typical cell walls in land plants are composed predominantly of polysaccharides, mainly cellulose microfibrils, which are embedded in a matrix of pectin and hemicellulose. Cellulose is synthesized by cellulose-synthesizing complexes localized on the plasma membrane, which spin out crystalline cellulose microfibrils. In contrast, hemicelluloses and pectins are synthesized in Golgi bodies, carried to the plasma membrane by vesicles, and secreted into the cell wall, where hemicelluloses cross-link with cellulose microfibrils and pectins fill the space in between to form the three dimensional dynamic structure of the cell wall.

The plant cell wall plays important roles in controlling cell differentiation. The thin wall of a young cell, called the primary cell wall, contains many enzymes, including not only glycosyl hydrolases, which simply disassemble cell wall polysaccharides, but also cell-wall modifying enzymes such as expansin, endoxyloglucan transferase/hydrolase, and pectinmethyl esterase, which are implicated in the remodeling of cell wall architecture, and are required for regulation of cell elongation and differentiation. After cell elongation ceases, the cell wall is generally thickened by formation of a secondary cell wall. Since cell walls connect cells to form tissues and provide the tissues with mechanical strength, they determine the growth and development of the entire plant body. Within the secondary cell wall is deposited lignin, which is essential in allowing land plants to strengthen vascular tissues. In addition, the cell wall works as an external sensor in defending against and responding to environmental stresses.

Cellulose is also the main component of algae and fungi cell walls. Compared to land plants, the process of cell wall formation can be more easily observed and analyzed in these organisms. Unicellular algae demonstrate a variety of shapes, which are determined by their primary cell walls according to the specialized accumulation of cell wall materials.

This chapter illustrates a variety of cell wall formations in fungal, algal, and plant cells. M. Osumi presents the excreted ribbon-like fibrillar network of glucan framing the cell wall in a reverting protoplast of fission yeast, *Schizosaccharomyces pombe*, by low-voltage scanning electron microscopy. M. Yamamoto and S. Kawano show cell wall formation during autosporeulation in the green alga *Chlorella vulgaris*, in which wall synthesis begins before successive protoplast division, using fluorescent microscopy as well as a rapid-freeze-fixation method and transmission electron microscopy. M. Yamamoto et al. also demonstrate four generations of cell walls in the green alga *Marvania geminata* by fluorescent microscopy and field emission scanning electron microscopy. S. Sekida and K. Okuda demonstrate the formation of a complex armor-like covering called the amphiesma, which is composed of a series of flattened vesicles containing cellulose plates, in the dinoflagellate *Scrippsiella hexapraecingula* using thin-section and freeze-fracture methods and electron microscopy, as well as fluorescent microscopy. T. Noguchi detects pectin in the primary cell wall of developing daughter semi-cells of *Micrasterias* by fluorescent microscopy. In addition, she shows cellulose-synthesizing rosettes in the plasma membranes of secondary cell walls forming *Micrasterias* and a *Closterium* zygote using freeze-fracture methods and electron microscopy. R. Yokoyama et al. demonstrate the immunological localization of two typical cell wall polysaccharides, pectin and β -1,3/1,4 mixed linked glucan, individually in two angiosperms, *Arabidopsis thaliana* and *Oryza sativa*, using fluorescent and electron microscopy.

The cell wall encloses each cell while at the same time enabling the transfer of solutes and signaling between cells via plasmodesmata. Casparian strips are a chemically modified region of the cell wall in the endodermis of vascular plant roots. The Casparian strip acts as a barrier that is thought to be crucial for selective nutrient uptake and exclusion of pathogens.

Y. Hayashi shows the distribution of plasmodesmata in the cell wall of cotyledons by osmium tetroxide-potassium ferricyanide staining and electron microscopy. Y. Honma and I. Karahara successfully show an isolated Casparian strip from a pea root and its meshwork structure by fluorescent microscopy.

T. Noguchi (✉)
Course of Biological Sciences, Faculty of Science, Nara Women's
University, Kitauoya-nishimachi, Nara 630-8506, Japan
e-mail: noguchi@cc.nara-wu.ac.jp

Plate 7.1

Ribbon-like fibrillar network of glucan in reverting *Schizosaccharomyces pombe* protoplast

Yeast, a typical fungus, has been used as a model system for various basic and applied fields of life science, medicine and biotechnology. Yeast sub-cellular structure is fundamentally the same as higher animal and plant cell structure. The cell wall is the sole yeast structure which is not found in animal cells. The cell wall is situated on the outer surface of the cell and is important for maintaining the genetically determined cell shape. The cell wall plays an important role in the transportation of materials into and out of the cell. The cell wall is the first line of defense of the host to a fungal infection from the standpoint of pathology, and in chemotherapy it is a primary target of antifungal agents. Analysis of the mechanisms of cell wall formation is therefore important in both basic and applied biology [1].

The ultrastructural image of the yeast cell was first described by Agar and Douglas in 1957 based on imaging of thin sections fixed with potassium permanganate (KMnO₄). Chemical fixation and embedding techniques present many problems in the preservation of membranous structures within yeast cells. Direct osmium tetroxide (OsO₄)-fixation results in poor preservation for all structures except protoplasts. Until the 1970s, only KMnO₄ was known to preserve yeast membrane architecture and show the membrane lipid bilayer with high electron density. In early ultrastructural studies of yeast, cells were fixed with KMnO₄ and embedded with methacrylate because OsO₄ and epoxy resin were found to not penetrate the thick cell walls. The first phase of yeast ultrastructural studies were in the 1960s, and organelles, especially mitochondria, were studied by single fixation with KMnO₄ or double fixation with GA-KMnO₄. In the 1970s GA-OsO₄ fixation was generally used following digestion of the cell wall by Zymolase (β-1, 3-glucanase). This method allows us to study yeast cells with rigid cell walls, which had been the principal obstacle to earlier physiological and morphological studies. In the 1980s the rapid freeze substitution technique was difficult to apply to yeast cells, but was modified by application of the 'sandwich method.' Although this technique is quite complicated, it allowed study of yeast cell ultrastructure at the same resolution level as is possible for other biological specimens [1].

This image is a low voltage scanning electron microscope (LVSEM) image of the ribbon-like fibrillar network of glucan framing the cell wall 5 h after it was regenerated from a reverting protoplast in fission yeast, *S. pombe*. In approximately 7 h these cells will revert to the original cylindrical

rod shape covered by galactomannan. This is the first image of a reverting protoplast photographed by LVSEM [2, 3].

Specimens were fixed with 2 % GA for 2 h, postfixed in 2 % OsO₄ for 1 h or with 2 % ruthenium tetroxide for 7 min, and prepared for SEM in the usual manner. Specimens were slightly coated with a 2 nm layer of platinum-carbon at 2×10^7 Pa and 10 °C in a Balzers 500 K with an electron gun and examined with the in-lens FESEM, Hitachi S-900, at 1–3 kV [3, 4]. Scale bar: 1 μm

Contributors

Masako Osumi*, Integrated Imaging Research Support, Villa Royal Hirakawa 103, 1-7-5-103 Hirakawa-Cho, Ciyoda-ku, Tokyo 102-0093, Japan

*E-mail: osumi@fc.jwu.ac.jp

References

1. Osumi M, (1998) The ultrastructure of yeast: cell wall structure and formation. *Micron* 29:207–233
2. Osumi M, Yamada N, Kobori H, Naito N, Baba M, Nagatani T (1989) Cell wall formation in regenerating protoplasts of *Schizosaccharomyces pombe*. *J Electron Microsc* 38:457–468
3. Osumi M (2012) Visualization of yeast cells by electron microscopy. *J Electron Microsc* 61:343–365
4. Osumi M, Yamada N, Yaguchi H, Kobori H, Nagatani T, Sato M (1995) Ultrahigh-resolution low-voltage SEM reveals ultrastructure of the glucan network formation fission yeast protoplast. *J Electron Microsc* 44:198–206

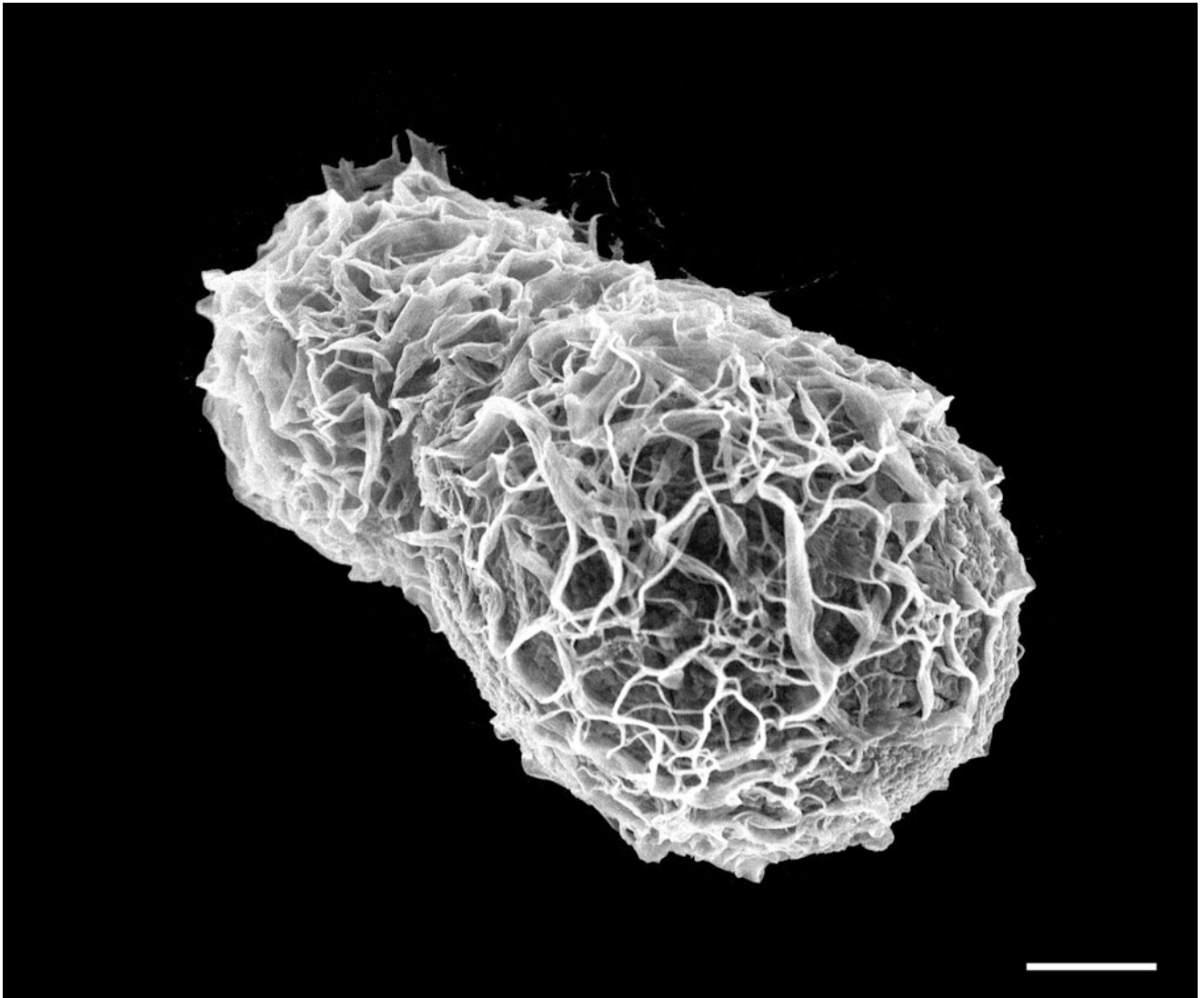


Plate 7.2

Mother and daughter cell walls during autospore formation in the green alga *Chlorella vulgaris*

Chlorella propagates by autospore formation, which is the formation of daughter cells with their own cell walls enveloping their protoplast. The structural changes which occur with growth of premature daughter cell walls during the mother cell-division phases in *C. vulgaris* were examined using the cell wall-specific fluorescent dye Fluostain I (A) and electron microscopy (B). Two clearly distinguishable stages of daughter cell wall synthesis are suggested by fluorescence observations: moderate synthesis occurs during the mother cell growth process and rapid synthesis occurs during the mother cell division phase. Daughter cell wall synthesis occurs over the cell surface in the early stage of the mother cell growth process, as indicated by electron microscopy studies. The newly synthesized daughter cell wall gradually becomes thick. After the successive second protoplast division, each daughter cell matured to a round shape. During the process of autospore maturation, the daughter cell wall (arrowhead) rapidly increased in thickness and reached substantially the thickness of the mother cell wall (double arrowhead) before hatching [5, 6].

For fluorescent microscopy of cell wall components with Fluostain I, cultured cells were stained with 0.001 % Fluostain I in PBS buffer. Stained samples were observed under ultraviolet excitation of Fluostain I using a fluorescence microscope. Stained cells were imaged by a chilled CCD camera system. For electron microscopy, cultured cells were fixed using the rapid freeze fixation method, followed by freeze substitution with 2.5 % glutaraldehyde in dry acetone. Samples were subsequently transferred to 2 % OsO₄ in dry acetone at 40 °C for 4 h. Ultra-thin sections were stained with 3 % uranyl acetate for 2 h at room temperature, with lead citrate for 10 min at room temperature, and finally examined with a transmission electron microscope at 100 kV. Scale bars: 5 μm (A), 500 nm (B). This figure is adapted from [5, 6].

References

5. Yamamoto M, Fujishita M, Hirata A, Kawano S (2004) Regeneration and maturation of daughter cell walls in the autospore-forming green alga *Chlorella vulgaris* (Chlorophyta, Trebouxiophyceae). *J Plant Res* 117:257–264
6. Yamamoto M, Kawano S (2004) Daughter cell wall synthesis in autospore-forming green alga, *Chlorella vulgaris* (IAM-C-536). *Cytologia* 69–3:i–ii

Contributors

Maki Yamamoto^{1*}, Shigeyuki Kawano², ¹Institute of Natural Sciences, Senshu University, 2-1-1 Higashimita, Tama, Kawasaki, Kanagawa 214-8580, Japan, ²Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Bldg. FSB-601, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan

*E-mail: yamamoto@isc.senshu-u.ac.jp

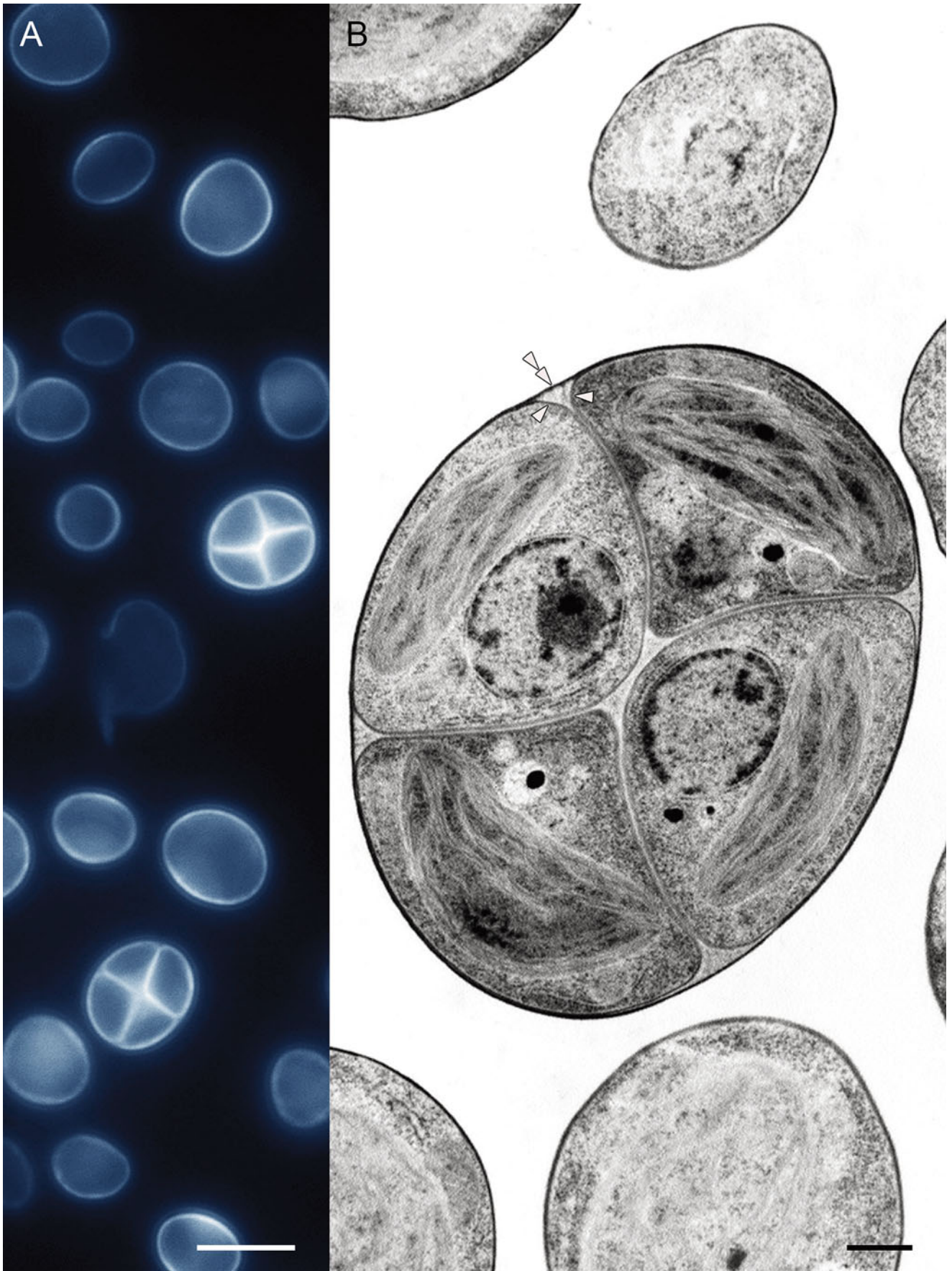


Plate 7.3

Great-grandmother, grandmother, mother, and daughter cell walls during budding in the green alga *Marvania geminata*

Marvania geminata propagates by budding. To examine structural changes in cell wall structure during the *M. geminata* cell cycle, we used Fluostain I, a cell wall-specific fluorescent dye (A–C), and field emission scanning electron microscopy (D). Cells in early growth phase were spherical (A), and Fluostain I fluorescence was observed at cell edges and in the upper and lower right-hand side of the image. Cells expanded by budding from the position of strong Fluostain I fluorescence to the right side of the image (B). During protoplast division, a belt of Fluostain I fluorescence was ascertained at the budding site (i.e., at the plane of division), which was the position of strong fluorescence during the growth phase. Subsequently, the two daughter cells separated (C) [7]. The daughter cell on the left side of the image is covered by great-grand mother (arrow), grandmother (triple arrowheads), mother (double arrowheads) and daughter cell walls (arrowhead); the daughter cell on the right side is covered by mother (double arrowheads) and daughter cell walls (arrowhead) (D). The two daughter cells were substantially asymmetrical from the stand of cell wall formation. Daughter cells entered the next cell cycle still retaining the mother and grandmother cell wall, causing the uncovered site of the cell to bud outward [8].

For fluorescence microscopy of cell wall components, cultured cells were stained with 0.001 % Fluostain I in PBS buffer. Stained samples were observed under ultraviolet excitation of Fluostain I using a fluorescence microscope. Samples were imaged with a chilled CCD camera system. For field emission scanning electron microscopy, cells were fixed with 2.5 % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h, washed three times (20 min × 3) and post-fixed with 1 % OsO₄ for 1 h at room temperature. To take away the mucilage around the *Marvania* cell wall, cells were treated with 5 % sodium hypochlorite for 10 min before postfixation. Cells were next washed two to six times for 15 min in Milli-Q water (Millipore, Billerica). Specimens were dehydrated with a graded ethanol series (30–100 %) followed by isoamyl acetate (50 % in ethanol and 100 %), and dried with a Hitachi critical point dryer (HCP-2; Hitachi). Specimens were coated with osmium using a Neoc osmium coater (Meiwafosis Co., Ltd), and imaged using an S-5000 field emission scanning electron microscope (Hitachi). Scale bars: 5 μm (C), 1 μm (D). This figure is adapted from [7, 8].

Contributors

Maki Yamamoto^{1*}, Satomi Owari², Shigeyuki Kawano³, ¹Institute of Natural Sciences, Senshu University, 2-1-1 Higashimita, Tama, Kawasaki, Kanagawa 214-8580, Japan, ²Neo-Morgan Laboratory Incorporated Research & Development, Biotechnology Research Center, 907 Nogawa, Miyamae-ku, Kawasaki, Kanagawa 216-0001, Japan, ³Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Bldg. FSB-601, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan
*E-mail: yamamoto@isc.senshu-u.ac.jp

References

- Yamamoto M, Nishikawa T, Kajitani H, Kawano S (2007) Patterns of asexual reproduction in *Nannochloris bacillaris* and *Marvania geminata* (Chlorophyta, Trebouxiophyceae). *Planta* 226:917–927
- Yamazaki T, Owari S, Ota H, Sumiya N, Yamamoto M, Watanabe K, Nagumo T, Miyamura S, Kawano, S (2013) Localoization and evolution of septins in algae. *Plant J* 74:605–614

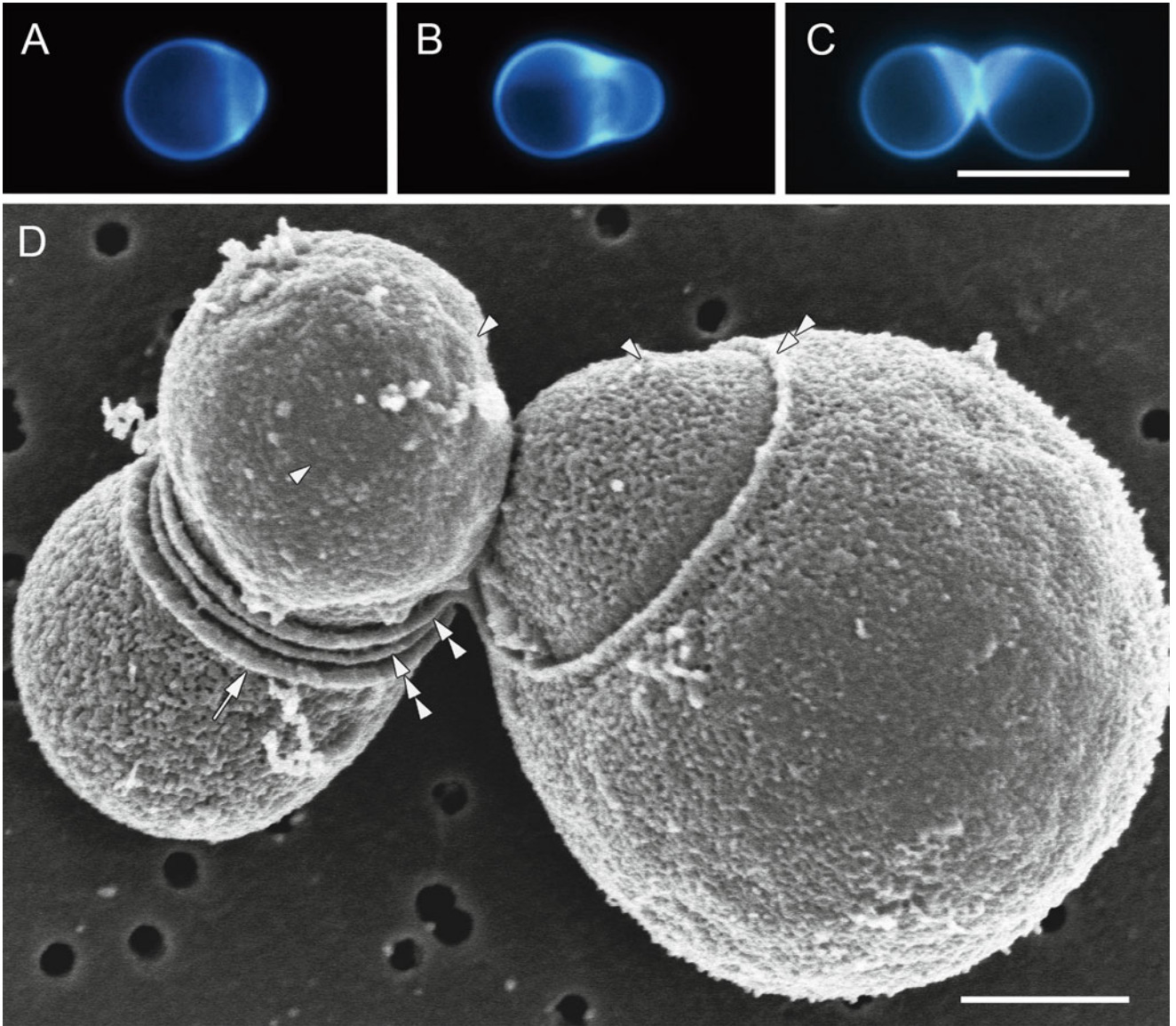


Plate 7.4

Formation of amphiesmal vesicles and thecal plates in the dinoflagellate *Scrippsiella hexapraeicingula*

Scrippsiella hexapraeicingula is an armored, peridinioid dinoflagellate that has a simple, asexual life cycle in which cultured cells alternate diurnally between motile and nonmotile forms. Motile cells transform into nonmotile cells through ecdysis. During the nonmotile phase, one or two daughter cells which develop new amphiesmal vesicles are produced inside the pellicle [9]. A cross section of a nonmotile cell fixed 6 h after ecdysis shows a thickened pellicle at the outside of the plasma membrane (pm) and developing amphiesmal vesicles (asterisks) with an outer amphiesmal vesicle membrane (arrow) and an inner amphiesmal vesicle membrane (double arrowheads) (A). Amphiesmal vesicles are empty but arranged in the same pattern as the developing thecal plates. Freeze-fracture images of nonmotile cells fixed 2 h (B) and 9 h (C) after ecdysis show the protoplasmic faces of outer amphiesmal vesicle membranes (oam PF) and exoplasmic faces of inner amphiesmal vesicle membranes (iam EF) and the plasma membrane (pm EF). Early amphiesmal vesicle-like patches developed within territories enclosed by broken lines in a cell 2 h after ecdysis (B), whereas adjacent almost complete amphiesmal vesicles came in contact with each other to form sutures (arrowheads) along the boundaries of territories in a cell 9 h after ecdysis (C) (adapted from [9]). Thecal plates are produced in the amphiesmal vesicles of motile cells [10], and therefore the thecal plate pattern is determined at the time of development of amphiesmal vesicles in nonmotile cells [11]. Motile cells were fixed and stained 5, 10, 15 and 30 min after they escaped from pellicles of nonmotile cells and began to swim (D). Incipient thecal plates appear first as groups of granular materials (blue) which increase in number, such that thin sheet-like thecal plates develop. Plate materials spread continuously within amphiesmal vesicles, and finally individual thecal plates become sufficiently close to each other to be arranged in a pattern specific to this species.

For thin sectioning, nonmotile cells were fixed by a cold block-freezing method. Freeze substitution with a 4:1 (v/v) mixture of acetone and methanol containing 2 % OsO₄ was carried out at -80 °C for 30 h. The sample was warmed gradually to room temperature, rinsed with ethanol, and embedded in LR White resin. For freeze fracture, nonmotile cells were frozen rapidly in nitrogen slush. Freeze fracture and metal shadowing were performed with a Baltec BAF 060 apparatus (Baltec Inc.) at -106 °C and 1 × 10⁻⁶ mbar. Specimens were shadowed unidirectionally at an angle of 60° with platinum-carbon and subsequently coated with

carbon to make replicas. For fluorescence microscopy, motile cells were fixed with seawater containing 1.2 % glutaraldehyde and 0.4 % OsO₄ at 4 °C for 10 min, rinsed with seawater, and stained with seawater containing 0.1 % Calcofluor White M2R (to stain thecal plate materials) and 1 µg/mL ethidium bromide (to stain nuclei). Samples were imaged with an epifluorescence microscope using ultraviolet excitation. Scale bars: 200 nm (A), 1 µm (B), 500 nm (C), 50 µm (D)

Contributors

Satoko Sekida, Kazuo Okuda*, Graduate School of Kuroshio Science, Kochi University, 2-5-1 Akebono-cho, Kochi 780-8520, Japan
*E-mail: okuda@kochi-u.ac.jp

References

- Sekida S, Horiguchi T, Okuda K (2001) Development of the cell covering in the dinoflagellate *Scrippsiella hexapraeicingula* (Peridinales, Dinophyceae). *Phycological Res* 49:163–176
- Sekida S, Horiguchi T, Okuda K (2004) Development of thecal plates and pellicle in the dinoflagellate *Scrippsiella hexapraeicingula* (Peridinales, Dinophyceae) elucidated by changes in stainability of the associated membranes. *Eur J Phycol* 39:105–114
- Sekida S, Takahira M, Horiguchi T, Okuda K (2012) Effects of high pressure in the armored dinoflagellate *Scrippsiella hexapraeicingula* (Peridinales, Dinophyceae): changes in thecal plate pattern and microtubule assembly. *J Phycol* 48:163–173

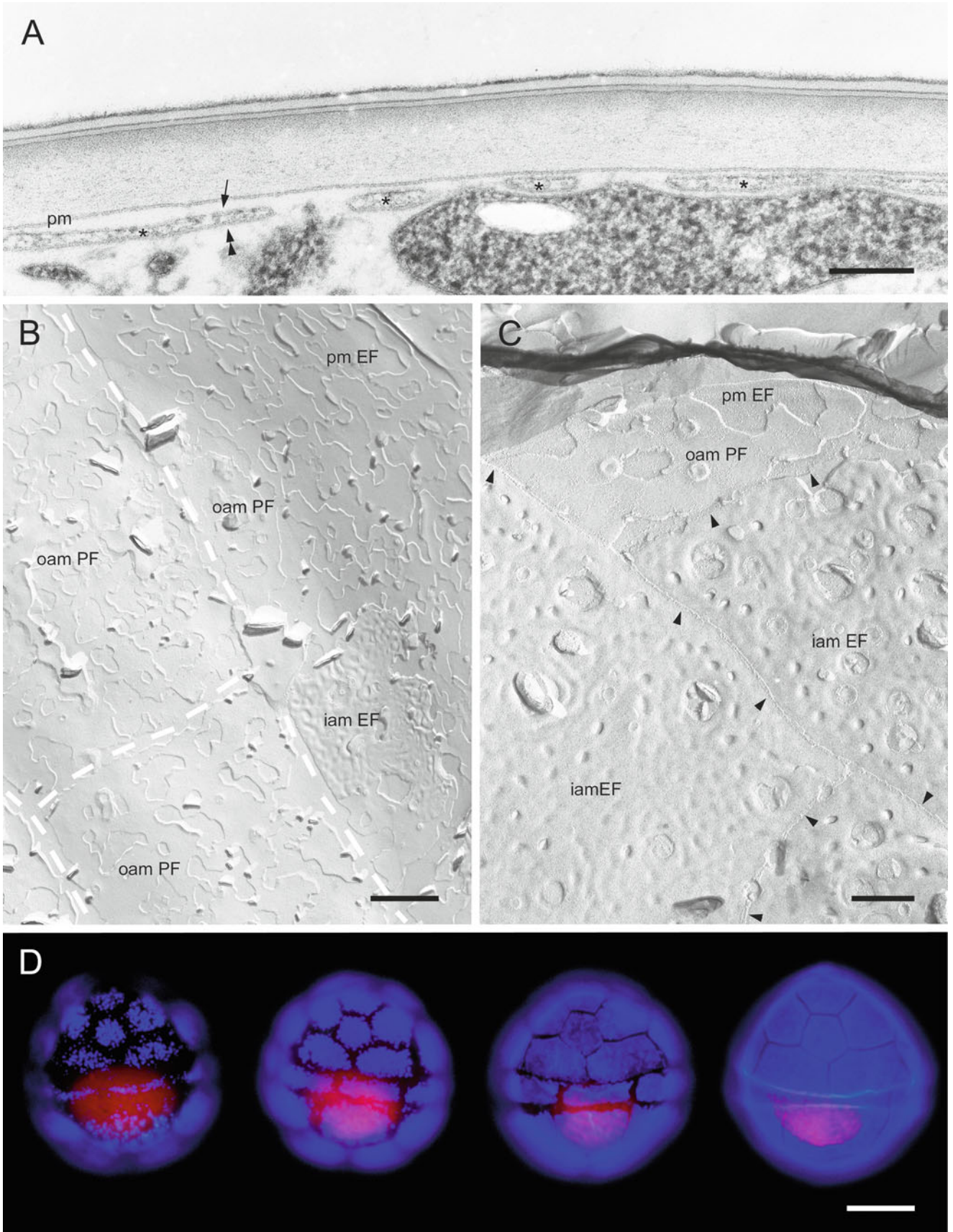


Plate 7.5

The elaborate shape of *Micrasterias* is formed by a primary cell wall containing pectin

The unicellular green algae *Micrasterias* is composed of two semi-cells joined at a deep median constriction. Each semi-cell has an elaborate outer shape composed of a number of lobes. The nucleus lies in the central isthmus defined by the median constriction. After mitosis, the cell is divided into two cells by a septum across the isthmus. Each daughter cell begins to grow as a hemispherical bulge, which later develops 3, 5, and then 9 lobes. The development of this elaborate cell has attracted the attention of many cell biologists as a model system for cell morphogenesis research.

The primary cell wall, which contains pectic substances, begins to grow after septum formation (upper cell) and continues until the daughter hemi-cell develops fully (lower cell). The main components of the primary cell wall are synthesized in Golgi bodies and carried by special vesicles which have a dark electron dense core and large vesicles when imaged by electron microscopy [12]. The primary wall is decomposed after the formation of the cellulosic secondary wall as shown by the weak fluorescence of the cell wall of mother semi-cells, which has also been confirmed by electron microscopy. Daughter cell shape is determined by the shape of the developed primary wall, which is induced by precocious differentiation of the wall at the sinus [13].

This figure is a fluorescent micrograph of growing *Micrasterias* sp. immunolabeled with an antibody against pectin purified from the green alga *Botryococcus braunii*.

Cells were fixed with 3 % paraformaldehyde, rinsed, treated with a monoclonal anti-pectin antibody, then treated with FITC-labeled anti-mouse IgG. Scale bar: 10 μm .

Contributors

Tetsuko Noguchi*, Course of Biological Sciences, Faculty of Science, Nara Women's University, Kitauoya-nishimachi, Nara 630-8506, Japan
*E-mail: noguchi@cc.nara-wu.ac.jp

References

12. Ueda K, Noguchi T (1976) Transformation of the Golgi apparatus in the cell cycle of a green alga, *Micrasterias americana*. *Protoplasma* 87:145–162
13. Ueda K, Yoshioka S (1976) Cell wall development of *Micrasterias americana*, especially in isotonic and hypertonic solutions. *J Cell Sci* 21:617–631

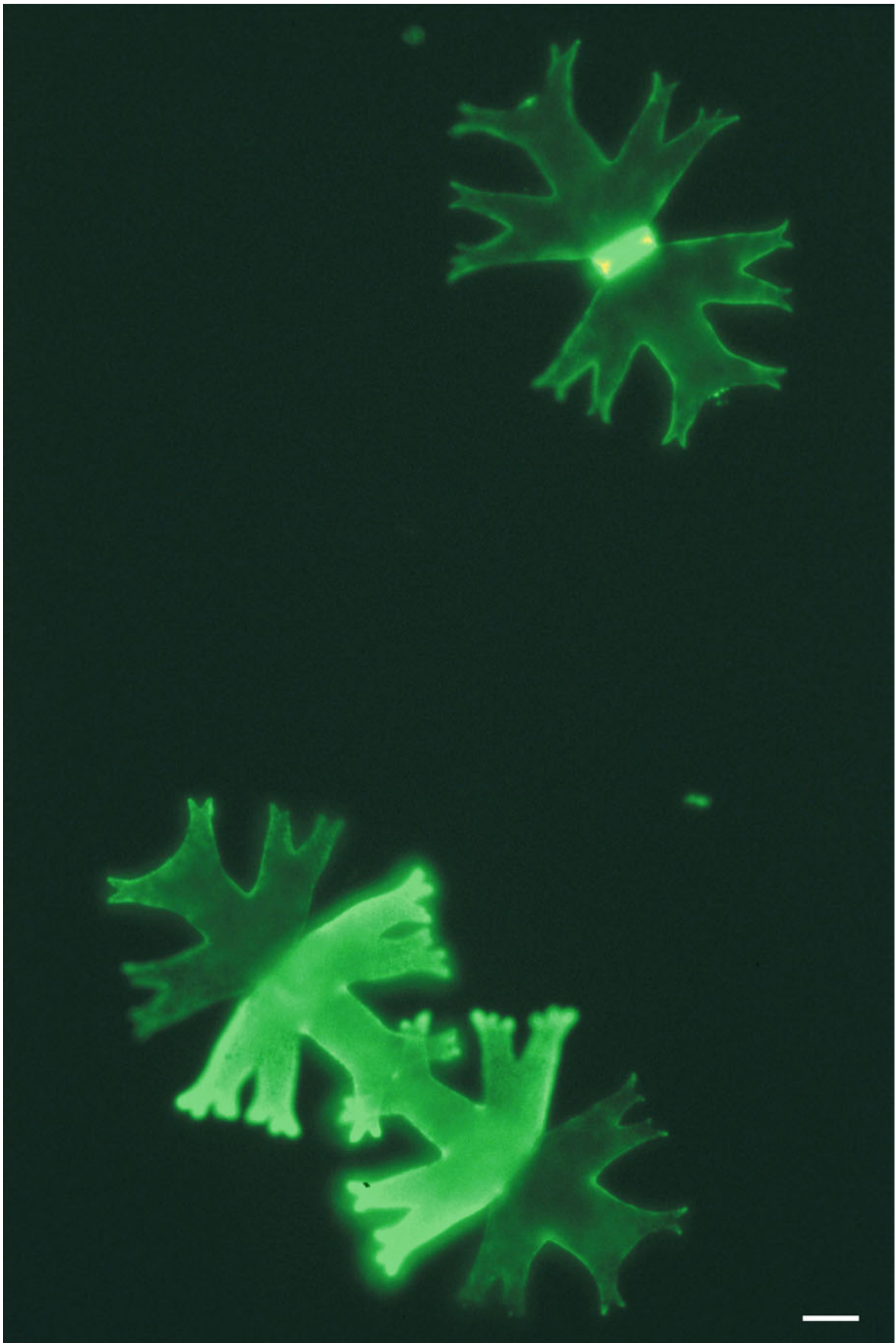


Plate 7.6

Cellulose-synthesizing rosettes in the green algae *Micrasterias* and *Closterium*

The secondary cell wall of *Micrasterias* is composed of cellulose microfibrils arranged in a parallel orientation that form layers of crossed microfibrils (right region in **(A)** adapted from [14]). The synthesis of the cellulosic secondary cell wall starts 6 h after daughter semi-cells are well developed (**(B)**, yellow; cellulosic secondary cell wall, red; chloroplast).

Freeze fracture electron microscopy allows visualization of the membrane interior, in which the fracture plane often passes through the hydrophobic interior of membrane lipid bilayers. In fully grown daughter semi-cells, the P-fracture face of the plasma membrane is composed of a hexagonal array of cellulose-synthesizing rosettes consisting of six particles (cellulose-synthases) **(C)**, which is never seen in the plasma membrane of non-growing cells **(A)** nor in those of mother semi-cells [14]. These cellulose-synthases are believed to be carried by special vesicles (flat vesicle) produced by Golgi bodies during the cellulose synthesis period. Hexagonal particle arrays and microfibril bands extended from these arrays are visible when cells are cultured in distilled water before freezing **(D)** adapted from [15]). Cellulose-synthesizing rosettes were discovered in *Micrasterias* [16].

The figures show transmission electron micrographs of freeze fracture replicas of *Micrasterias crux-melitensis*, a zygote accumulating cellulose wall layers of *Closterium reinhardii*, and a fluorescence micrograph of growing *M. crux-melitensis* stained with calcofluor.

For freeze fracture, living cells mounted on supporting copper disks were frozen in liquid propane. Fracturing and shadowing were carried out at $-112\text{ }^{\circ}\text{C}$. Cleansed replicas were imaged with an electron microscope at 100 kV. For fluorescence microscopy, cells were stained with 0.02 % calcofluor. Scale bars: 250 nm (**(A)**, **(C)**, **(D)**), 100 μm (**(B)**).

References

14. Noguchi T, Tanaka K, Ueda K (1981) Membrane structure of dictyosomes, large vesicles and plasma membranes in a green alga, *Micrasterias crux-melitensis*. Cell Struct Funct 6:217–229
15. Noguchi T, Ueda K (1985) Cell walls, Plasma membranes, and dictyosomes during zygote maturation of *Closterium ehrenbergii*. Protoplasma 128:64–71
16. Giddings Jr TH, Brower D, Staehelin LA (1980) Visualization of particle complexes in the plasma membrane of *Micrasterias denticulate* associated with the formation of cellulose fibrils in primary and secondary cell walls. J Cell Biol 84:327–339

Contributors

Tetsuko Noguchi*, Course of Biological Sciences, Faculty of Science, Nara Women's University, Kitaouya-nishimachi, Nara 630-8506, Japan

*E-mail: noguchi@cc.nara-wu.ac.jp

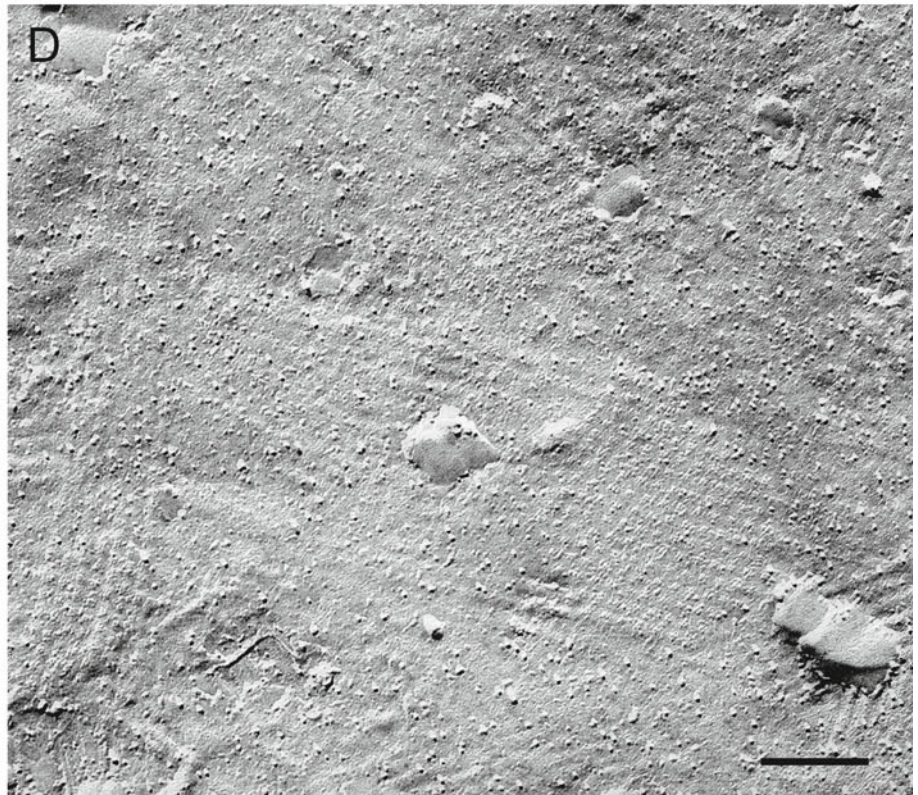
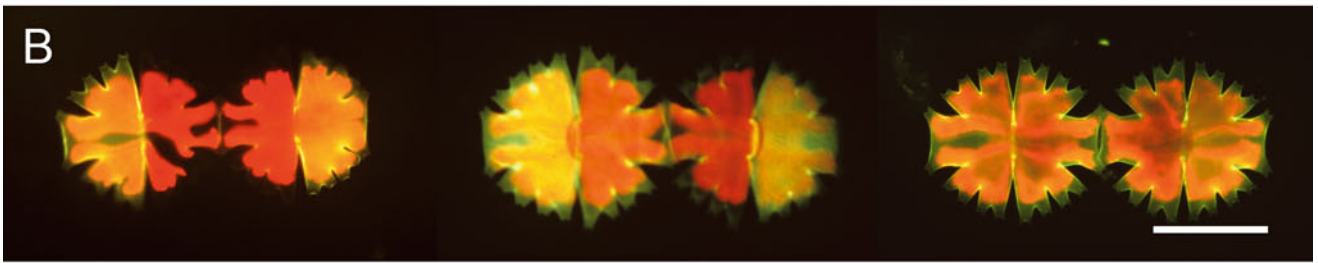


Plate 7.7

Localization of typical cell wall polysaccharides pectin and β -1,3/1,4 mixed linkage glucan in *Arabidopsis thaliana* and *Oryza sativa*

The structural and chemical nature of plant cell walls diversified extensively during the terrestrial invasion of plants in the Devonian period. The cell walls of land plants are characterized by two distinct layers of cell walls: the primary cell wall and the secondary cell wall. The former is ubiquitously found in all land plants, whereas the latter is found exclusively in vascular plants and is deposited onto the primary cell wall in specific tissue types, namely vascular tissues, after cell expansion has ceased.

The chemical nature of the primary cell wall has diversified among angiosperms. Although pectin and xyloglucans are abundant in the cell walls of eudicotyledonous plants, such as *Arabidopsis thaliana* (L.) Heynh [17], they are less abundant in the cell walls of Poales species, such as rice (*Oryza sativa* L.). Rice cell walls contain higher levels of β -1,3/1,4 mixed linkage glucans (MLG) and arabinoxylans than xyloglucans and pectin.

Pectin in the *A. thaliana* inflorescence stem (A) and MLG in *O. sativa* leaf blades (B) are shown by immunofluorescence. Green fluorescence indicates the presence of pectin or MLG. Similarly, pectin in cortical cells of the *A. thaliana* inflorescence stem (C) and MLG in *O. sativa* collenchyma cells of the leaf blade (D) are shown by electron microscopy. Black (electron dense) particles indicate the presence of pectin or MLG within the cell wall.

For immunofluorescence analysis, stems or leaf blades were cross-sectioned using a vibratome at a thickness of 70 μ m and fixed with 4 % paraformaldehyde in 20 mM sodium cacodylate buffer, pH 7.4, followed by probing with JIM5 monoclonal antibody for pectin or anti-MLG monoclonal antibody. JIM5 antibody specifically binds to a sparsely methylated or unesterified homogalacturonan domain of pectin, while the MLG antibody recognizes linear (1,3/1,4)- β -oligosaccharide segments in MLG. To enable highly sensitive immunodetection, the tyramide signal amplification method was used. For immunogold labeling, segments of stems or the leaf blades were fixed for 1 h in 0.1 M phosphate buffer, pH 7.2, containing 4 % paraformaldehyde and 0.05 % glutaraldehyde, then embedded in LR White resin (Sigma) and polymerized by heat. Fixed samples were subjected to ultra-thin sectioning followed by immuno-gold labeling with the appropriate monoclonal antibody for pectin or MLG.

Cor, cortex; Ph, phloem; If, interfascicular fiber; Ep, epidermal cell; Pa, parenchyma; Col, collenchyma. Scale bars: 100 μ m (A), 30 μ m (B), 5 μ m (C), 1 μ m (D).

Contributors

Ryusuke Yokoyama, Hideki Narukawa, Kazuhiko Nishitani*, Laboratory of Plant Cell Wall Biology, Graduate School of Life Sciences, Tohoku University, 6-3 Aoba, Aramaki, Aoba-Ku, Sendai, Miyagi 980-8578, Japan

*E-mail: nishitan@m.tohoku.ac.jp

References

- Hongo S, Sato K, Yokoyama R, Nishitani K (2012) Demethylesterification of the primary wall by ECTIN METHYLESTERASE35 provides mechanical support to the *Arabidopsis* stem. *Plant Cell* 24:2624–2634

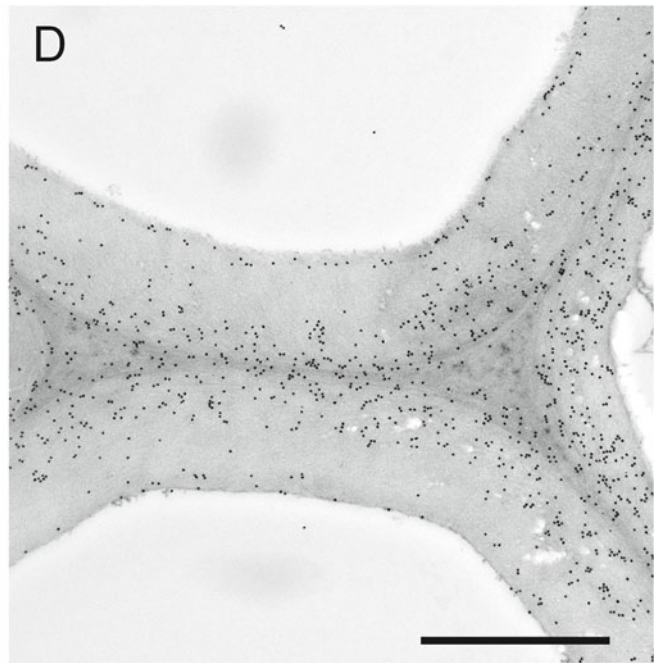
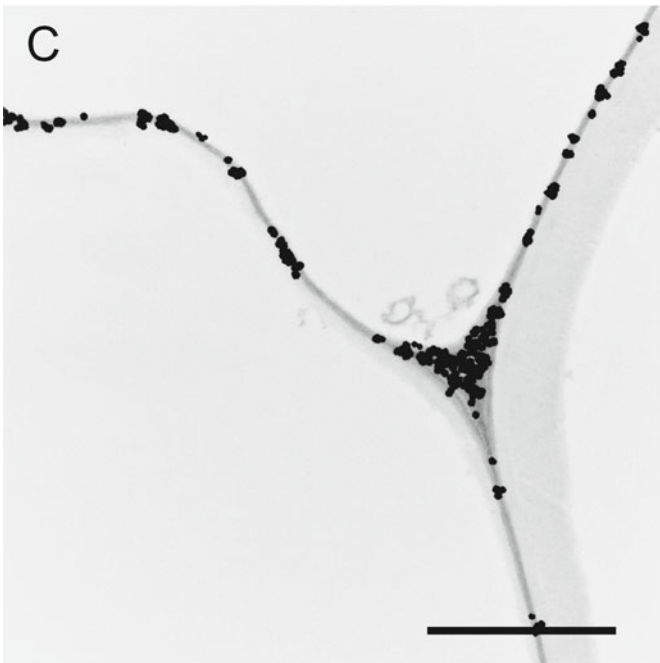
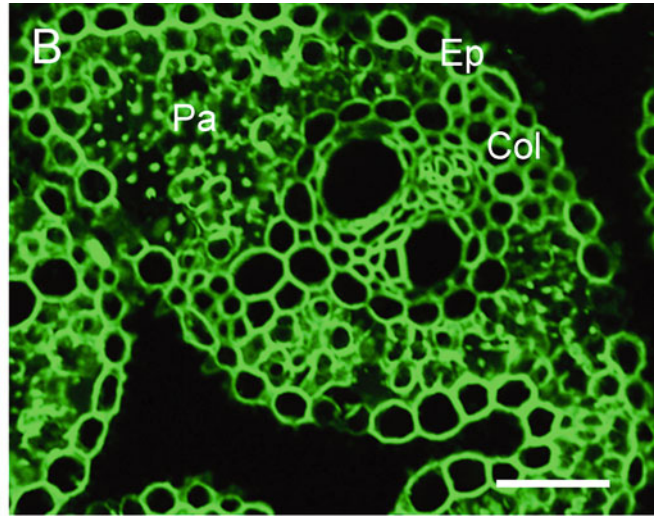
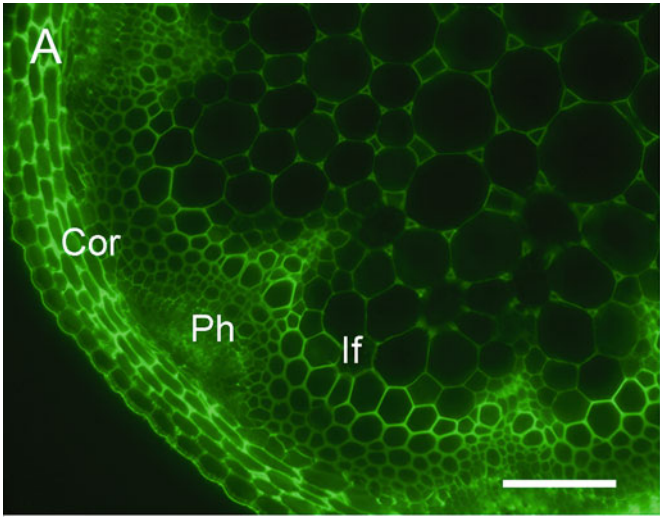


Plate 7.8

Plasmodesmata directly connect the cytoplasm of neighboring plant cells

Plant cells are surrounded by cell walls. Together, a pair of cell walls and the intervening lamella form an extracellular domain which separates neighbouring cells. Plasmodesmata penetrate both the primary and secondary cell walls, allowing transport of molecules between adjacent cells by symplast. Plasmodesmata also play important roles in cellular communication.

Membranes were stained by the osmium tetroxide-potassium ferricyanide method (A). Plasmodesmata are tubular connections, 50–60 nm in diameter at the midpoint between adjacent cells [18]. The plasma membrane is continuous from one cell to the next cell at plasmodesmata. Plasmodesmata contain a desmotubule, which is a narrow tube-like structure (arrow heads) and is continuous with the smooth endoplasmic reticulum (ER) in the connected cells (A). A vertical cell wall section is imaged in samples prepared by high-pressure freezing and freeze substitution (B). Cortical microtubules can be clearly seen under the plasma membrane (arrows). The desmotubule does not fill the plasmodesma completely (B). The cytoplasmic sleeve exists between the plasma membrane and the desmotubule (arrow heads). Trafficking of molecules and ions through plasmodesmata occurs through this sleeve. Smaller molecules and ions can easily pass through plasmodesmata by diffusion without requiring additional chemical energy. Larger molecules, including proteins and RNA, can also pass through the cytoplasmic sleeve. It is known that in some cases molecule size restrictions can be overcome, though the mechanism of these transport systems is not yet understood. Special proteins and some viruses are able to increase the diameter of the channels enough for unusually large molecules to pass through [19]. A typical plant cell may have 10^3 to 10^5 plasmodesmata connecting to adjacent cells, which equates to 1 to 10 plasmodesmata per μm^2 . There are two forms of plasmodesmata: primary plasmodesmata, which form during cell division when parental endoplasmic reticulum are trapped in the new cell wall [20], and secondary plasmodesmata, which form between mature cells. Members of the *Charophyceae*, *Charales*, *Coleochaetales* and *Phaeophyceae* algal families, as well as all embryophyte land plants, have plasmodesmata.

To stain ER and desmotubule, 5-day-old cotyledons were fixed in cacodylate buffer (Ph 7.4) containing 4 % paraformaldehyde, 1 % glutaraldehyde, and 0.1 M CaCl_2 for 3 h at 4 °C, washed with 0.1 M cacodylate buffer for 1.5 h, postfixed with 2 % OsO_4 plus 0.8 % $\text{K}_3\text{Fe}(\text{CN})_6$ and 1 μM CaCl_2 in 0.1 M cacodylate buffer for 2 h at room

temperature, and serially dehydrated in ethanol. To visualize desmotubules in the cell wall, cotyledons were frozen with a high-pressure freezer, then dehydrated for 2 days at -85 °C in acetone containing 3 % (w/v) osmium tetroxide. Samples were embedded in Spurr resin, ultrathin sectioned, stained with uranium and lead, and imaged with an electron microscope. Scale bars: 1 μm .

Contributors

Yasuko Hayashi*, Department of Environmental Science, Graduate School of Science and Technology, Niigata University, Ikarashi, Niigata 950-2181, Japan

*E-mail: yhayashi@env.sc.niigata-u.ac.jp

References

18. Ding B, Turgeon R, Parthasarathy MV (1992) Substructure of freeze-substituted plasmodesmata. *Protoplasma* 169:28–41
19. Ding B (1998) Intercellular protein trafficking through plasmodesmata. *Plant Mol Biol* 38:279–310
20. Ehlers K, Kollmann R (2001) Primary and secondary plasmodesmata: structure, origin, and functioning. *Protoplasma* 216:1–30

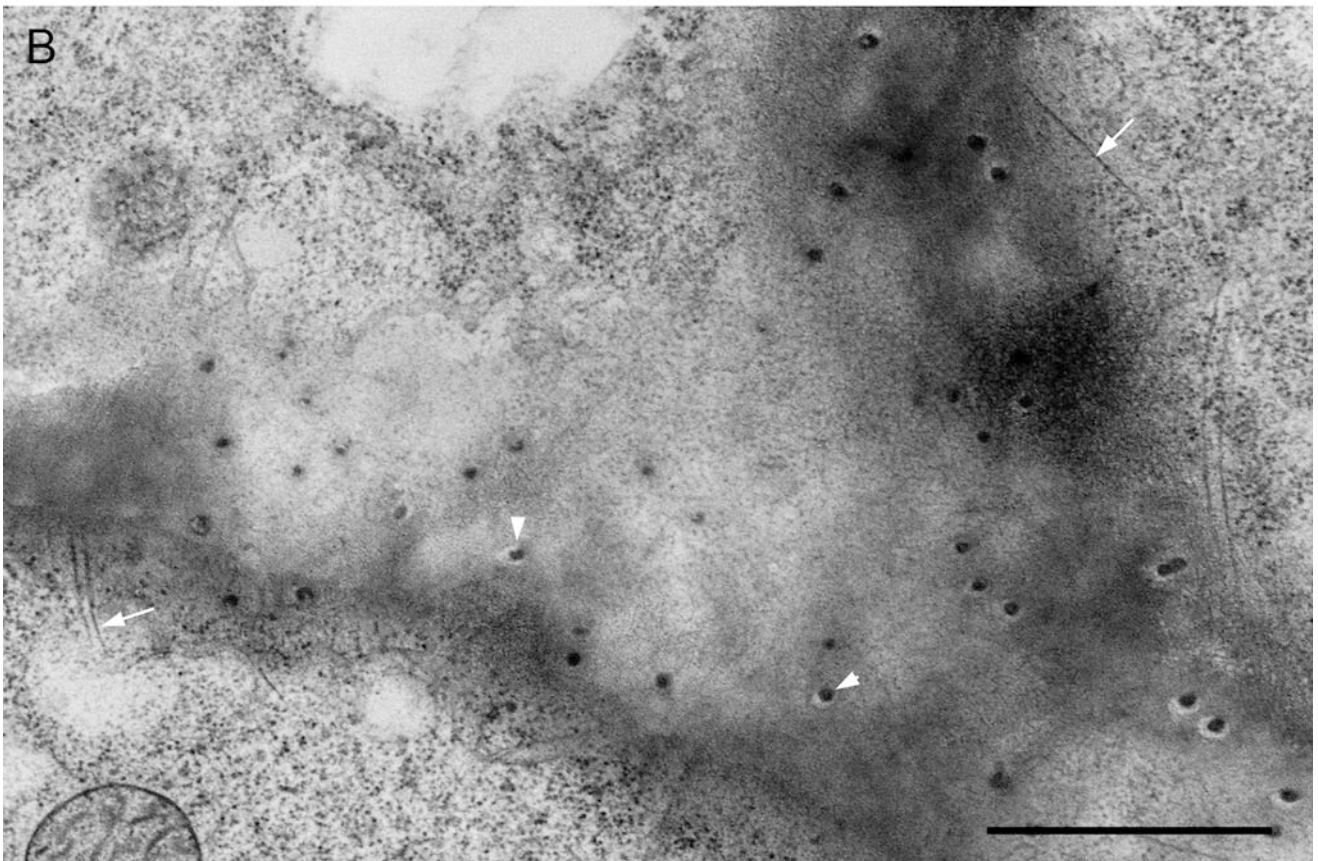
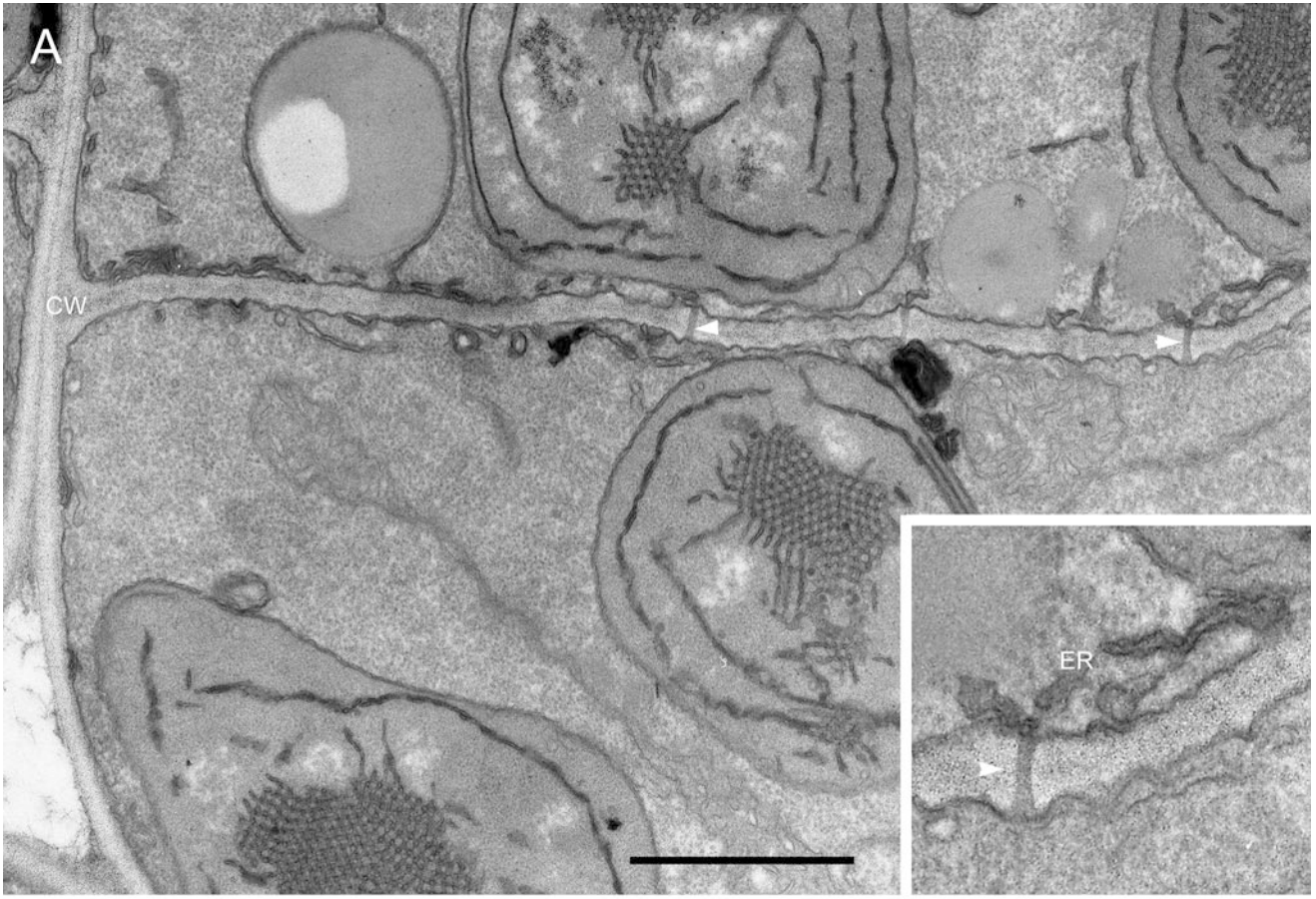


Plate 7.9

Meshwork structure of the Casparian strip

The Casparian strip is the barrier to apoplastic transport located in the radial walls of endodermal cells in roots and shoots of vascular plants. The Casparian strip is formed by individual endodermal cells, but must be continuous to function. The Casparian strip encircles each endodermal cell and is in fact continuous throughout the entire endodermal tissue. There is morphological evidence that, prior to lignification, positional information in the radial wall of endodermal cells defines the future site of strip formation.

This figure shows the meshwork structure of the Casparian strip. The main panel shows the hypocotyl of a 7 days old *Arabidopsis* seedling (*Arabidopsis thaliana* (L.) Heynh., Col-0) grown in the light (A). The Casparian strip and xylem vessels emit autofluorescence under UV light because they are impregnated with lignin (A). Three dimensional models of the Casparian strip in a hypocotyl display the tissue organization of a hypocotyl (B), the endodermis and xylem vessels (C), and the Casparian strip and xylem vessels (D). A Casparian strip isolated from a pea root (*Pisum sativum* L. cv. Alaska) observed under a fluorescence microscope is also shown (E adapted from [21]).

Isolation of the Casparian strip was performed as follows. Pea roots were split in half and incubated with a solution of cell wall digesting enzymes. Vascular tissues were removed from split roots and endodermal layers were picked up with forceps. Cells attached to isolated endodermal layers were removed and washed. Isolated endodermal layers were agitated on a Vortex mixer to remove any cytoplasm attached to the Casparian strips. *Arabidopsis* hypocotyls were fixed in FAA (3.7 % formaldehyde, 5 % acetic acid, and 50 % ethanol) and cleared in 10 % (w/v) KOH (105 °C, 1 min) (A–D adapted from [22]). Cleared hypocotyls were mounted on a glass slide and observed with a fluorescent microscope (BX-FLA; Olympus) equipped with a filter assembly for excitation by ultraviolet (UV) light (U-MWU: excitation filter, BP330-385; dichroic mirror, DM-400). Scale bar: 50 μm (A).

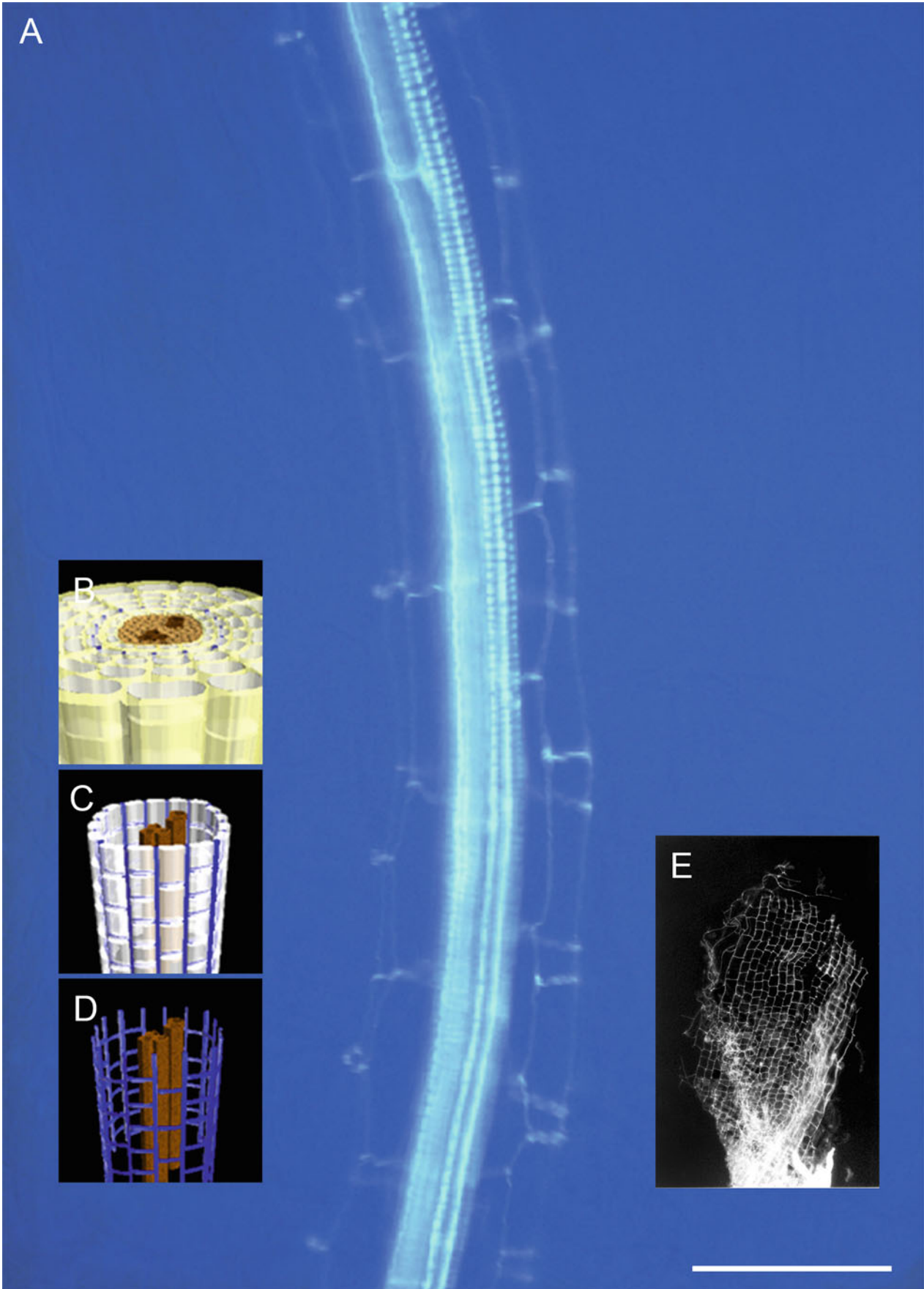
References

21. Karahara I (1995) The Casparian strip: the tight junction of plants. *Kagaku to Seibutsu* 33:246–251 (Japanese)
22. Homma Y, Karahara I (2004) The Casparian strip in a cleared hypocotyl of *Arabidopsis*. *Cytologia* 69:i–ii (Technical note)

Contributors

Yoshihiro Honma, Ichirou Karahara*, Department of Biology, Graduate School of Science and Engineering, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan

*E-mail: karahara@sci.u-toyama.ac.jp



Chapter References

1. Osumi M (1998) The ultrastructure of yeast: cell wall structure and formation. *Micron* 29:207–233
2. Osumi M, Yamada N, Kobori H, Naito N, Baba M, Nagatani T (1989) Cell wall formation in regenerating protoplasts of *Schizosaccharomyces pombe*. *J Electron Microsc* 38:457–468
3. Osumi M (2012) Visualization of yeast cells by electron microscopy. *J Electron Microsc* 61:343–365
4. Osumi M, Yamada N, Yaguchi H, Kobori H, Nagatani T, Sato M (1995) Ultrahigh-resolution low-voltage SEM reveals ultrastructure of the glucan network formation fission yeast protoplast. *J Electron Microsc* 44:198–206
5. Yamamoto M, Fujishita M, Hirata A, Kawano S (2004) Regeneration and maturation of daughter cell walls in the autospore-forming green alga *Chlorella vulgaris* (Chlorophyta, Trebouxiophyceae). *J Plant Res* 117:257–264
6. Yamamoto M, Kawano S (2004) Daughter cell wall synthesis in autospore-forming green alga, *Chlorella vulgaris* (IAM-C-536). *Cytologia* 69–3:i–ii
7. Yamamoto M, Nishikawa T, Kajitani H, Kawano S (2007) Patterns of asexual reproduction in *Nannochloris bacillaris* and *Marvania geminata* (Chlorophyta, Trebouxiophyceae). *Planta* 226:917–927
8. Yamazaki T, Owari S, Ota H, Sumiya N, Yamamoto M, Watanabe K, Nagumo T, Miyamura S, Kawano S (2013) Localization and evolution of septins in algae. *Plant J* 74:605–614
9. Sekida S, Horiguchi T, Okuda K (2001) Development of the cell covering in the dinoflagellate *Scrippsiella hexapraeicingula* (Peridinales, Dinophyceae). *Phycological Res* 49:163–176
10. Sekida S, Horiguchi T, Okuda K (2004) Development of thecal plates and pellicle in the dinoflagellate *Scrippsiella hexapraeicingula* (Peridinales, Dinophyceae) elucidated by changes in stainability of the associated membranes. *Eur J Phycol* 39:105–114
11. Sekida S, Takahira M, Horiguchi T, Okuda K (2012) Effects of high pressure in the armored dinoflagellate *Scrippsiella hexapraeicingula* (Peridinales, Dinophyceae): changes in thecal plate pattern and microtubule assembly. *J Phycol* 48:163–173
12. Ueda K, Noguchi T (1976) Transformation of the Golgi apparatus in the cell cycle of a green alga, *Micrasterias americana*. *Protoplasma* 87:145–162
13. Ueda K, Yoshioka S (1976) Cell wall development of *Micrasterias americana*, especially in isotonic and hypertonic solutions. *J Cell Sci* 21:617–631
14. Noguchi T, Tanaka K, Ueda K (1981) Membrane structure of dictyosomes, large vesicles and plasma membranes in a green alga, *Micrasterias crux-melitensis*. *Cell Struct Funct* 6:217–229
15. Noguchi T, Ueda K (1985) Cell walls, plasma membranes, and dictyosomes during zygote maturation of *Closterium ehrenbergii*. *Protoplasma* 128:64–71
16. Giddings Jr TH, Brower D, Staehelin LA (1980) Visualization of particle complexes in the plasma membrane of *Micrasterias denticulate* associated with the formation of cellulose fibrils in primary and secondary cell walls. *J Cell Biol* 84:327–339
17. Hongo S, Sato K, Yokoyama R, Nishitani K (2012) Demethylesterification of the primary wall by PECTIN METHYLESTERASE35 provides mechanical support to the Arabidopsis stem. *Plant Cell* 24:2624–2634
18. Ding B, Turgeon R, Parthasarathy MV (1992) Substructure of freeze-substituted plasmodesmata. *Protoplasma* 169:28–41
19. Ding B (1998) Intercellular protein trafficking through plasmodesmata. *Plant Mol Biol* 38:279–310
20. Ehlers K, Kollmann R (2001) Primary and secondary plasmodesmata: structure, origin, and functioning. *Protoplasma* 216:1–30
21. Karahara I (1995) The Casparian strip: the tight junction of plants. *Kagaku to Seibutsu* 33:246–251 (Japanese)
22. Homma Y, Karahara I (2004) The Casparian strip in a cleared hypocotyl of Arabidopsis. *Cytologia* 69:i–ii (Technical note)