Chikako Nagasato*, Makoto Terauchi, Atsuko Tanaka and Taizo Motomura

Development and function of plasmodesmata in zygotes of *Fucus distichus*

Abstract: Brown algae have plasmodesmata, tiny tubular cytoplasmic channels connecting adjacent cells. The lumen of plasmodesmata is 10–20 nm wide, and it takes a simple form, without a desmotubule (the inner membrane structure consisting of endoplasmic reticulum in the plasmodesmata of green plants). In this study, we analyzed the ultrastructure and distribution of plasmodesmata during development of *Fucus distichus* zygotes. The first cytokinesis of zygotes in brown algae is not accompanied by plasmodesmata formation. As the germlings develop, plasmodesmata are found in all septal cell walls, including the first cell division plane. Plasmodesmata are formed *de novo* on the existing cell wall. Pit fields, which are clusters of plasmodesmata, were observed in germlings with differentiated cell layers. Apart from the normal plasmodesmata, these pit fields had branched plasmodesmata that appeared to arise from the lateral preexisting ones. Fluorescent tracers with different molecular sizes were microinjected to examine the size exclusion limit of molecules for transit through the plasmodesmata. Fluorescein isothiocyanate (FITC)-dextran of 3 kDa size was spread over the germlings, and 10 kDa FITC-dextran was tracked only in the rhizoid. The size exclusion limit was <10 kDa for the thallus but <40 kDa for the rhizoid.

Keywords: brown algae; cell differentiation; microinjection; pit field; plasmodesmata.

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***Corresponding author: Chikako Nagasato,** Muroran Marine Station, Field Science Center for Northern Biosphere, Hokkaido University, Muroran 051-0013, Japan,
e-mail: nagasato@fsc.hokudai.ac.jp
Makoto Terauchi: Research Center for Inland Seas, Kobe University, Kobe 657-8501, Japan
Atsuko Tanaka and Taizo Motomura: Muroran Marine Station, Field Science Center for Northern Biosphere, Hokkaido University, Muroran 051-0013, Japan

Introduction

Multicellular organisms such as animals, fungi, land plants, and brown algae have specific cellular connections. These structures connect the cytoplasm of adjacent cells and provide a route for cell-cell communication. Green plants, including land plants and certain species of green algae (reviewed in Robards and Lucas 1990, Raven 1997), and brown algae (Bisalputra 1966) possess plasmodesmata. These are cytoplasmic canals that pass through the septal cell wall and connect adjacent cells. Plasmodesmata in green plants and brown algae share similar characteristics; however, plasmodesmata in brown algae lack a desmotubule, a tubular strand of connecting endoplasmic reticulum (ER) that penetrates the plasmodesmata of land plants (Terauchi et al. 2012). The diameter of the plasmodesmata in land plants is 20–50 nm, but in brown algae, it is 10–20 nm.

Intercellular connections in land plants are divided into primary and secondary plasmodesmata based on the time of their formation (reviewed in Ehlers and Kollmann 2001, Burch-Smith et al. 2011). Primary plasmodesmata are formed during cytokinesis as simple straight forms, whereas secondary plasmodesmata arise in an existing cell wall, irrespective of cytokinesis. Formation of the primary plasmodesmata in land plants has been well documented (Hepler 1982). The ER is trapped in the growing cell plate, eventually forming the plasmodesmata. Secondary plasmodesmata have simple, branched, or twined forms (Ehlers and Kollmann 2001, Burch-Smith et al. 2011). The occurrence of these structures is divided into "*de novo*" and "modified" types. *De novo* secondary plasmodesmata are simple structures indistinguishable from the primary plasmodesmata, and thus, their formation is difficult to monitor. Modified secondary plasmodesmata have branching forms of Y-, V-, X-, and H-shapes or a twined form (Ehlers and Kollmann 2001, Burch-Smith et al. 2011). Branched plasmodesmata are formed by lateral branching from the preexisting simple plasmodesmata (Burch-Smith et al. 2011), whereas twined plasmodesmata are formed by insertion of new plasmodesmata into existing ones during cell wall expansion (Faulkner et al. 2008). Furthermore, the H-shaped plasmodesmata are considered to be formed

by the development of a central cavity connecting twined plasmodesmata. In addition, branched plasmodesmata may form twined simple plasmodesmata intermediates (Burch-Smith et al. 2011). The presence of secondary plasmodesmata is correlated with growth, development, and response to extracellular signals.

In brown algae, a new septal cell wall is created by growth of a new cell partition membrane and deposition of cell wall components (Nagasato and Motomura 2002, Nagasato et al. 2010, 2014). Although plasmodesmata formation in brown algae has been observed in the cytokinesis of vegetative cells, previous studies have shown that the first cytokinesis in zygotes of brown algae is not accompanied by the formation of plasmodesmata. During cytokinesis in the apical meristem of *Dictyota dichotoma*, plasmodesmata formation is initiated by invaginations of the membranous sacs, transitional membranous structures in the formation of a new cell partition membrane (Terauchi et al. 2012), which penetrate the membranous sacs. The region of newly formed plasmodesmata during cytokinesis later becomes a pit field (Terauchi et al. 2012). Recently, we reported that the complexity of body plan in brown algae influences the number and arrangement of plasmodesmata (Terauchi et al. 2015). The distribution of plasmodesmata was examined in brown algal species showing uniseriate and multiseriate filamentous thalli and complex multicellular thalli and was compared between sporophytes (complex multicellular thalli) and gametophytes (uniseriate filament) of *Saccharina japonica*. The plasmodesmata in uniseriate and multiseriate filamentous brown algae were found to be sparsely distributed, whereas species with complex multicellular thallus had pit fields (Terauchi et al. 2015). This pattern was also found in the alternation of generations of *S. japonica*. It is expected that plasmodesmata probably occur secondarily in the first cell division plane, and pit fields in species with complex multicellular thallus are formed alongside cellular differentiation. However, in brown algae, the existence of secondary plasmodesmata and the occurrence of pit fields during development in the complex multicellular species have not been confirmed until now.

In green plants, inorganic ions, metabolites, RNAs, and proteins are transported through plasmodesmata (Lucas et al. 1995, McLean et al. 1997, Kim et al. 2005). The size exclusion limit (SEL), which has been measured by microinjection of fluorescent tracers with different sizes is 1–3 kDa, depends on the species and cell types (Erwee and Goodwin 1983, Goodwin 1983, Terry and Robards 1987, Kempers et al. 1993, Radford and White 2001, 2011). Molecular movement and SEL in plasmodesmata are regulated by a divalent ion such as Ca^{2+} (Erwee and Goodwin 1983) and by degeneration/synthesis of callose deposited at the constricted neck region of the plasmodesmata (Radford and White 2001, Zavaliev et al. 2011, 2013). In brown algae, symplastic transport has been elucidated using radioisotopes (Schmitz and Lobban 1976, Amat and Srivastava 1985). However, the maximum size of molecules that can pass through plasmodesmata and the regulation system for transport, such as that described in land plants, remain unclear. To our knowledge, only one study has been conducted on the movement of the fluorescent tracer through the plasmodesmata by microinjection in the fucoid zygotes of *Fucus spiralis* (Bouget et al. 1998), showing that 10 kDa molecules could pass through the plasmodesmata in *Fucus* germlings.

In this study, we observed the ultrastructure of *Fucus distichus* at each developmental stage, in order to resolve open questions about brown algal plasmodesmata, the presence of secondary plasmodesmata, and the occurrence of pit fields. Moreover, to understand the function of plasmodesmata, we investigated the SEL of plasmodesmata by microinjecting fluorescent tracers with different molecular sizes.

Materials and methods

Culture

Mature thalli of the monoecious species *Fucus distichus* Linnaeus were collected at Oinaoshi, Muroran, Hokkaido, Japan (42°30′N, 140°97′E), from April to June 2014. The receptacles were washed with filtered seawater and wiped with paper towels. They were placed in Petri dishes and kept overnight at 18°C under continuous light from fluorescent lamps at 30–40 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Before the release of eggs and sperm, the receptacles were transferred to a cold, dark place for 2 h. The release of eggs and sperm and fertilization were performed as described in previous studies (Abe 1970, Wakana and Abe 1992, Motomura 1994). Cooled seawater was poured into dishes containing the receptacles to induce the release of gametes and fertilization. The zygotes were set on a gold carrier (flat specimen carrier, Leica Microsystems, Wetzlar, Germany) for high-pressure freezing or on a glass slide with a TaKaRa slide seal (Takara, Shiga, Japan) and incubated at 18°C with Provasoli's enriched seawater (PES) medium (Provasoli 1968) containing 40 $mg \cdot l^{-1}$ chloramphenicol prior to microinjection.

High-pressure freezing and electron microscopy

Zygotes on the golden carrier were frozen in a Leica EM PACT2 high-pressure freezer (Leica Microsystems) at -180°C, 2100 bar, and kept in liquid nitrogen. Frozen samples were transferred to a freeze substitution medium (acetone containing 2% osmium tetroxide) in liquid nitrogen and stored at -85°C for about 2 days. Next, the samples were kept at -20°C for 2 h and then transferred to 4°C for additional 2 h. Finally, the temperature of the fixative was allowed to rise gradually to room temperature. The samples were then washed several times with acetone at room temperature and embedded in Spurr's epoxy resin on dishes of aluminum foil. Serial sections were cut using a diamond knife on an RMC MT-X ultramicrotome (RMC, Tucson, AZ, USA) and mounted on formvar-coated slot grids. Sections were stained with TI blue (Nisshin EM Co. Ltd, Tokyo, Japan) and lead citrate and observed using an electron microscope (JEM-1011, JEOL Ltd., Tokyo, Japan).

Microinjection

Zygotes on the glass slide were incubated under 18°C in a continuous unidirectional light to re-orient the direction of the rhizoid growth. Ten days after fertilization, the rhizoid region divided uniseriately into several cells. Microinjection was performed into a rhizoid cell near the end of the thallus region. Micromanipulators (combination of a coarse manipulator, MN-4, and a three-axis joystick oil hydraulic micromanipulator, MO-202; Narishige, Tokyo, Japan) were attached to an Olympus IX71 inverted microscope (Olympus, Tokyo, Japan). The glass capillaries (GDC-1; Narishige) were pulled using a microcapillary Faller MCF-100 (Nepa Gene Co., Ltd., Tokyo, Japan). To assess the SEL of plasmodesmata, fluorescein isothiocyanate (FITC) conjugated dextran of different sizes (3 kDa, 10 kDa, and 40 kDa; Invitrogen-Molecular Probes, Carlsbad, CA, USA) were used. Fluorescent tracers were diluted to 1 mg·ml^{-1} with injection solution (0.2 mM KCl, 10 mM HEPES, 0.55 M mannitol, pH 7.0; Farnham et al. 2013). Capillaries were filled with a tracer, ionic liquid (SW-25, Nepa Gene Co., Ltd.), a laser absorbent and UV absorbent (WF-25, Nepa Gene Co., Ltd.). Micromanipulation was performed by using a laser thermal microinjector LTM (Nepa Gene). The injected germlings were observed 2 days later under an epifluorescence microscope (BX50W-FL; Olympus) equipped with differential interference contrast (DIC). Photographs were taken using a CCD camera (AxioCam; Zeiss, Oberkochen, Germany), and digital images were converted with AxioVision 4.8 (Zeiss) and Photoshop Elements 10 (Adobe Systems, San Jose, CA, USA) for the final image presentation.

Results

Zygotes of *Fucus distichus* underwent the first cell division only 22 h after fertilization (Figure 1A), followed by the second cell division 4 h later when kept at 18°C and under continuous light. The first division plane was always

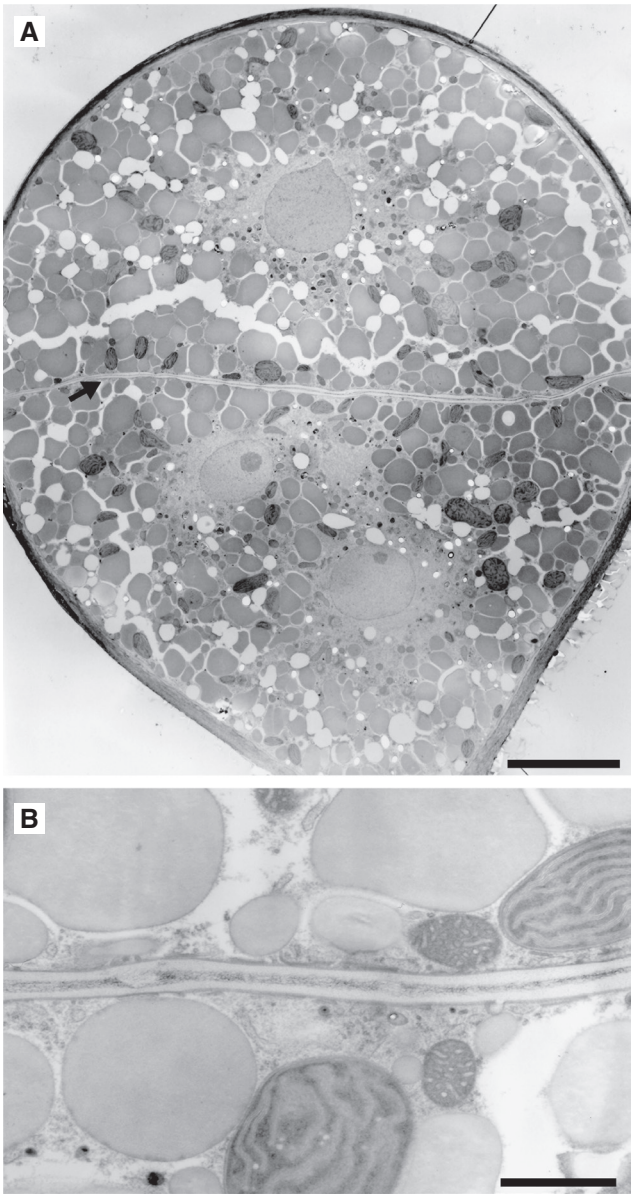

Figure 1: The first cell division of a zygote of *Fucus distichus*. (A) Whole zygote. Arrow indicates the first division plane. (B) First cell division plane. Scale bars=10 μm (A), 1 μm (B).

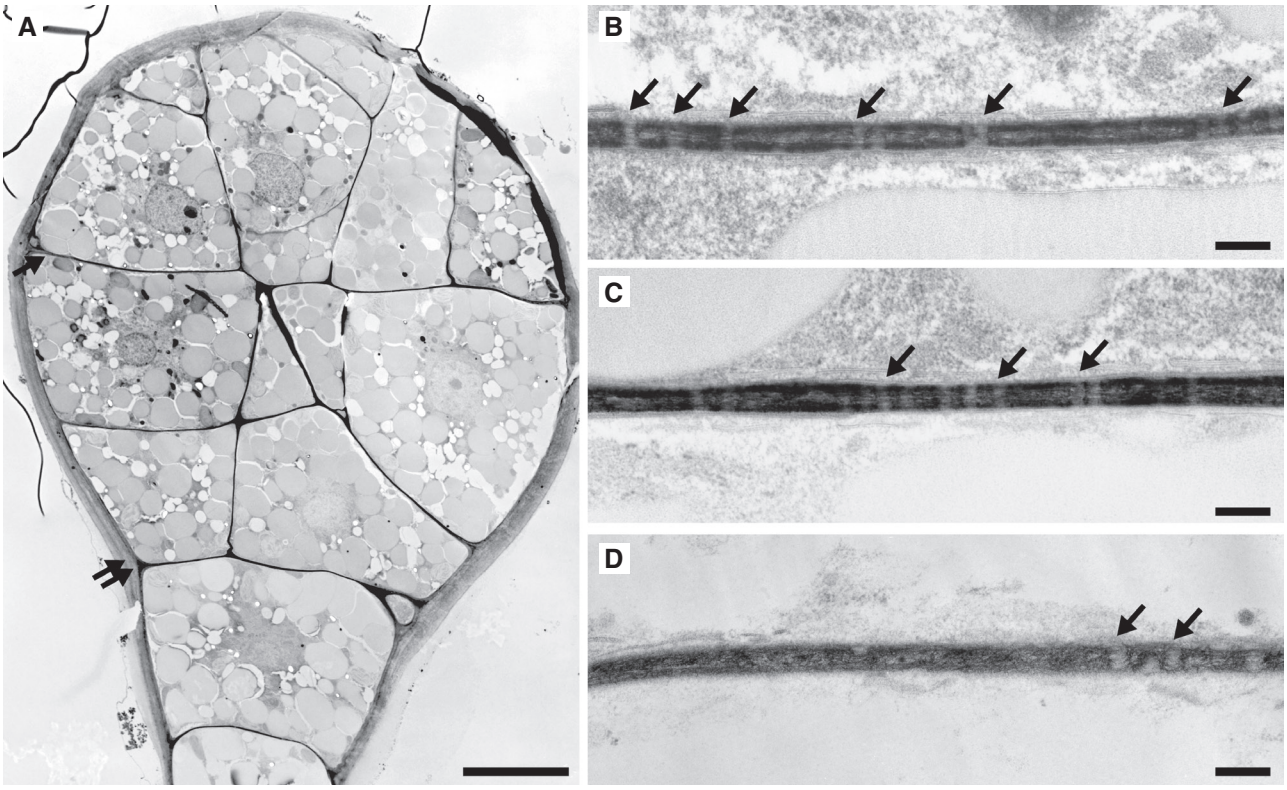

Figure 2: Germling of *Fucus distichus* 3 days after fertilization. (A) Overview of thallus and part of rhizoid. Arrow indicates the first division plane, and double arrows the border between thallus and rhizoid. (B) First cell division plane. Arrows indicate plasmodesmata. (C) Border between thallus and rhizoid. (D) Septum cell wall in the rhizoid tip. Scale bars=10 μm (A), 200 nm (B–D).

perpendicular to the longitudinal axis of the zygote, dividing it into a thallus and rhizoid side. At that time, plasmodesmata in the first cell division plane were not seen (Figure 1B). The second cell division occurred simultaneously in the thallus and rhizoid cells. In the thallus cell, the cell division plane was parallel to the growth axis, but the cell division plane in the rhizoid was perpendicular to the growth axis (Figure 2A). We examined the presence of plasmodesmata in all septal cell walls in young sporophytes 3 days after fertilization. We also examined the plasmodesmata distribution pattern in the first cell division plane (Figure 2B, arrow in Figure 2A), in the border zone between thallus and rhizoid (Figure 2C, double arrows in Figure 2A), and in the rhizoid (Figure 2D). Plasmodesmata were present sparsely in each cell wall. The diameter of the cell wall pores around the plasmodesmata in the first cell division plane, in the cell wall between thallus and rhizoid, in the upper part of the rhizoid cell, and in the tip of the rhizoid were 43 nm (n=10), 45 nm (n=15), 48 nm (n=13), and 66 nm (n=14), respectively. The diameter of the cell wall pores in the rhizoid tip was significantly larger than in the upper part of the rhizoid ($p<0.01$).

We also examined the distribution of plasmodesmata in a 5-day-old germling (Figure 3). In all cells, the nuclei were located in the center, and the cell components were almost identical (Figure 3A). There was no difference in plasmodesmata of the cells in the thallus. The longitudinal and transverse sections of plasmodesmata are shown in Figure 3B–D. The frequency of plasmodesmata on the septal cell walls increased compared to their frequency in the 3-day-old zygotes. However, the pit fields were not clearly detected.

Cellular differentiation started 10 days after fertilization (Figure 4A). The intensity of staining of individual cellular components differed between outer and inner layers of cells. The inner cells contained larger vacuoles than the outer ones (Figure 4B), whereas vacuoles containing phenolic compounds occupied the cells of the outer layer, which resulted in poor infiltration of resin (Figure 4B). The plasmodesmata in each septal cell wall were concentrated in one place, forming a "pit field" (Figure 4C). The distance between the centers of adjacent plasmodesmata was measured from the images showing the cross section of plasmodesmata. The plasmodesmata were, on average, spaced at 77 nm (n=76). This value was smaller than in the 5-day-old germling (125 nm, n=31; Figure 3D). The density of plasmodesmata within the cell wall increased with the maturation of pit

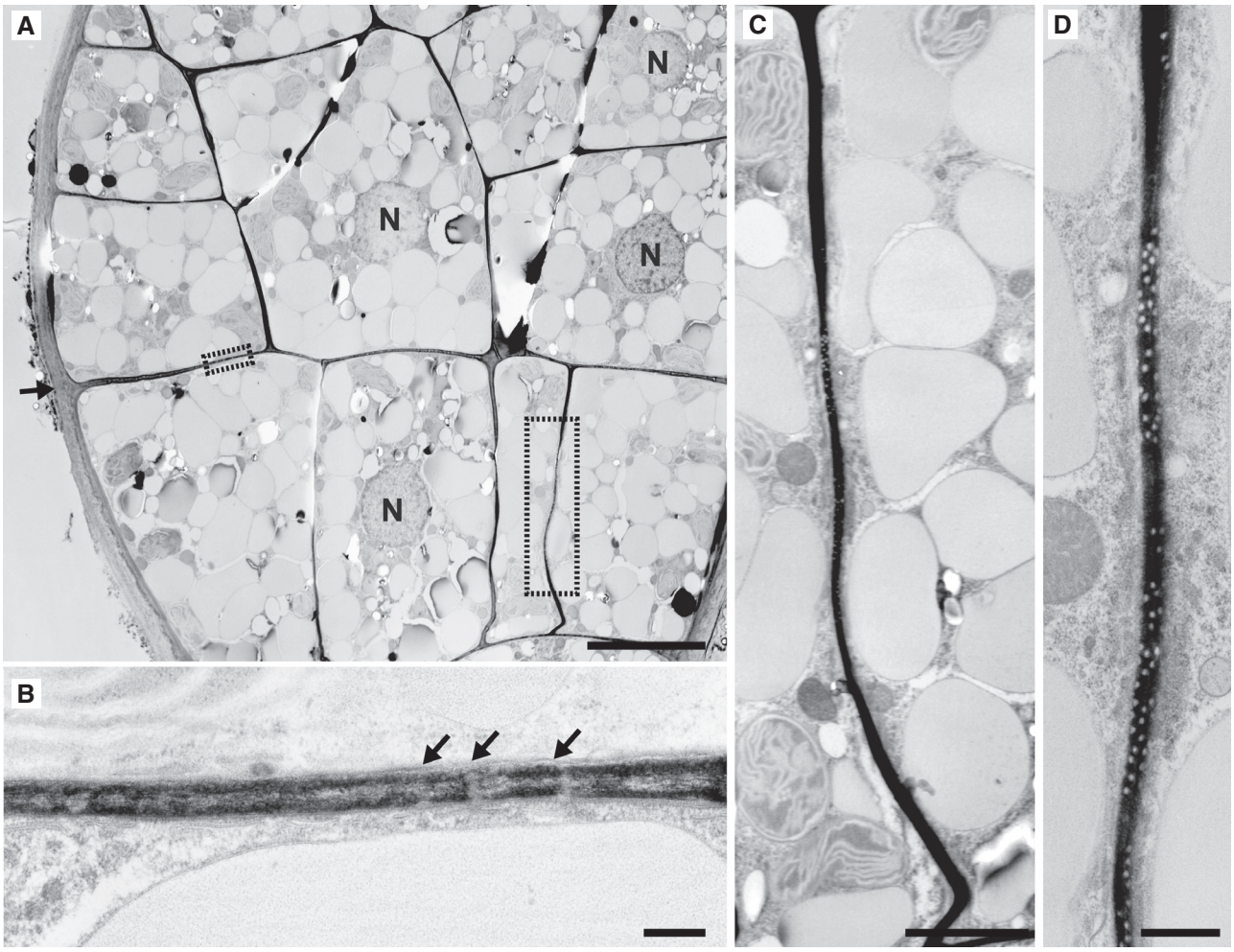

Figure 3: Germling of *Fucus distichus* 5 days after fertilization. (A) Part of thallus. Nuclei (N) are seen in the centers of cells. Arrow indicates first cell division plane. Rectangles indicate areas shown in (B–D). (B) Longitudinal section of plasmodesmata. Arrows show plasmodesmata. (C) Transverse section of plasmodesmata. (D) Magnified image of (C). Scale bars=10 μm (A), 200 nm (B), 2 μm (C), 500 nm (D).

fields. The pit fields were positioned in the middle or near the ends of the cell walls (Figure 4D,E). In the outer cell layer, pit fields were observed in the regions adjacent to the inner cells (Figure 4B). Frequently, pit fields were divided in two by an orthogonal cell wall (Figure 4D,E). The orthogonal cell wall was a new septal cell wall that formed in the middle part of a pit field in the mother cell wall (Figure 4E). As a result, the daughter cells shared one pit field.

Mature thalli of *F. distichus* have medulla (Figure 5A), cortex, and meristoderm layers (Figure 5B). Pit fields were detected in the septal cell walls of all tissue layers (Figure 5A–C). Regions of the cell wall without pit fields were thicker than those with pit fields (Figure 5C). The average diameter of the cell wall pores around plasmodesmata was 56 nm (n=85). We examined the detailed structure of plasmodesmata in a pit field from consecutive serial sections (Figure 5D–G). Most plasmodesmata in the pit field appeared to have a simple cylindrical form, but Y-shaped (branched) plasmodesmata were also found (Figure 5D,F,H,I), which were probably modified from pre-existing simple plasmodesmata.

Microinjection using fluorescent tracers with different molecular sizes is a useful tool to examine cell-to-cell transportation of molecules. To minimize the damage to cells caused by injection, we used a laser-assisted thermal-expansion microinjection system. In this system, a fine needle (<0.5 μm) is applied to administer the fluorescent tracers. All injected cells survived in this analysis. Dextran-conjugated FITC of sizes 3, 10 and 40 kDa were injected into the *Fucus* germlings 10 days after fertilization. The number of germlings showing dye movement and the number of performed injections is indicated in Table 1. After 2 days, the 3-kDa tracer had moved all over the germlings (Figure 6A,B), whereas the 10-kDa tracer had passed through only the rhizoid region (Figure 6C,D).

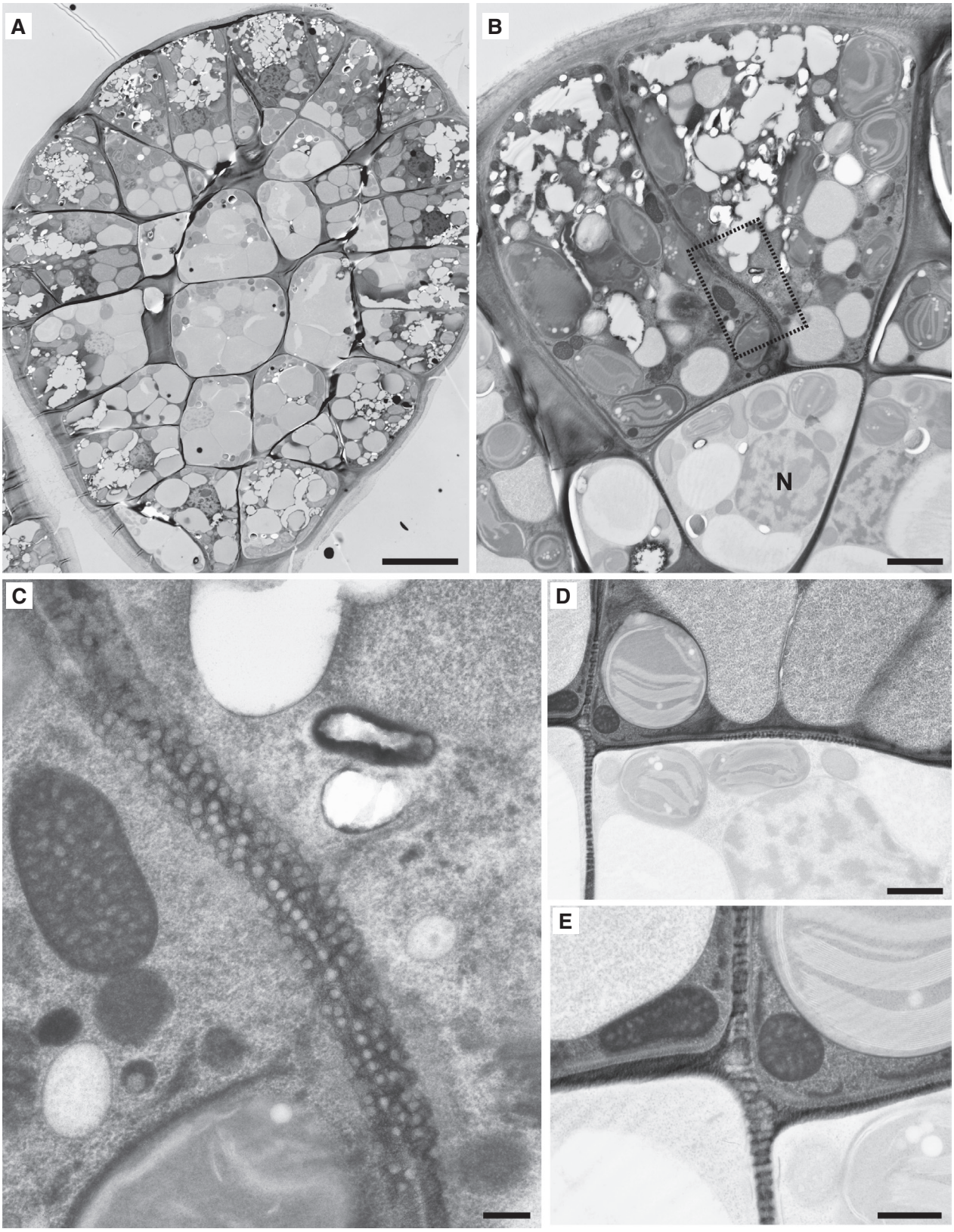

Figure 4: Sporophyte of *Fucus distichus* 10 days after fertilization. (A) Overview of thallus. Beginning of differentiation of cell layers can be seen. (B) Outer and inner cell layers. N, nucleus. Rectangle indicates area of wall with pit field. (C) Enlarged image of pit field in cross wall between cells of outer layer (rectangle in B). (D) Pit fields in cell wall between outer and inner cell layers. (E) Pit field divided in two by an orthogonal cell wall. Scale bars=10 μm (A), 2 μm (B), 200 nm (C), 1 μm (D), 500 nm (E).

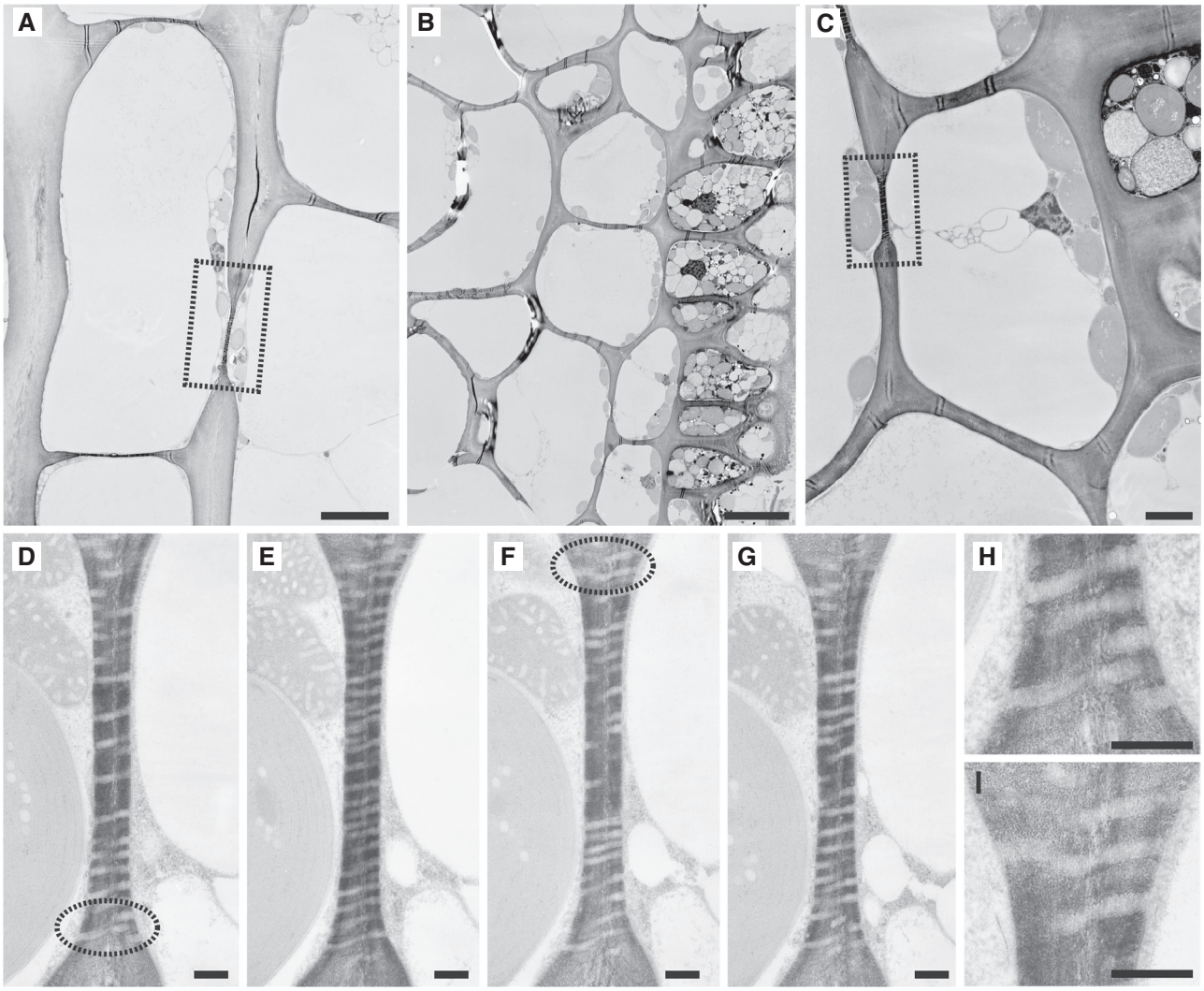

Figure 5: Mature sporophyte of *Fucus distichus* from the field. (A) Medulla layer. Large vacuoles occupy centers of cells, and other organelles are appressed to the edge. Rectangle indicates area with pit field. (B) Cortex and meristoderm layers. Cells in the cortex contain vacuoles with different contents. (C) Pit field in wall between cortex cells (indicated by rectangle). (D–G) Consecutive serial sections of pit field shown in (C). Ovals in (D) and (F) indicate branched plasmodesmata. (H) Branched plasmodesmata from (D). (I) Branched plasmodesmata from (F). Scale bars=5 μm (A), 10 μm (B), 2 μm (C), 200 nm (D–G), 500 nm (H, I).

In the case of the 40-kDa tracer, the molecules remained in the injected cell (Figure 6E,F). These results indicate that the SEL differs between the thallus and rhizoid regions.

Table 1: Cell-to-cell movement of fluorescent probes microinjected into rhizoid cells of 10-day-old sporophytes of *Fucus distichus*.

Size of probes	Germlings showing dye movement	Injected cells
3 kDa	26[a]	26
10 kDa	22[b]	22
40 kDa	0	26

[a]Fluorescent tracer observed all over the sporophyte.
[b]Movement of probes was restricted to the rhizoid.

Discussion

This study is the first report showing secondary plasmodesmata in brown algae. There were two origins in the occurrence of secondary plasmodesmata, *de novo* formation in the existing cell walls and the lateral branching from simple ones. It is suggested that secondary plasmodesmata would be formed also in other brown algae. The possible reasons for the absence of previous reports of secondary plasmodesmata, including branched plasmodesmata, in brown algae (Terauchi et al. 2015) are as follows: secondary plasmodesmata are indistinguishable from primary plasmodesmata, the frequency of secondary plasmodesmata is low, and their formation is limited to

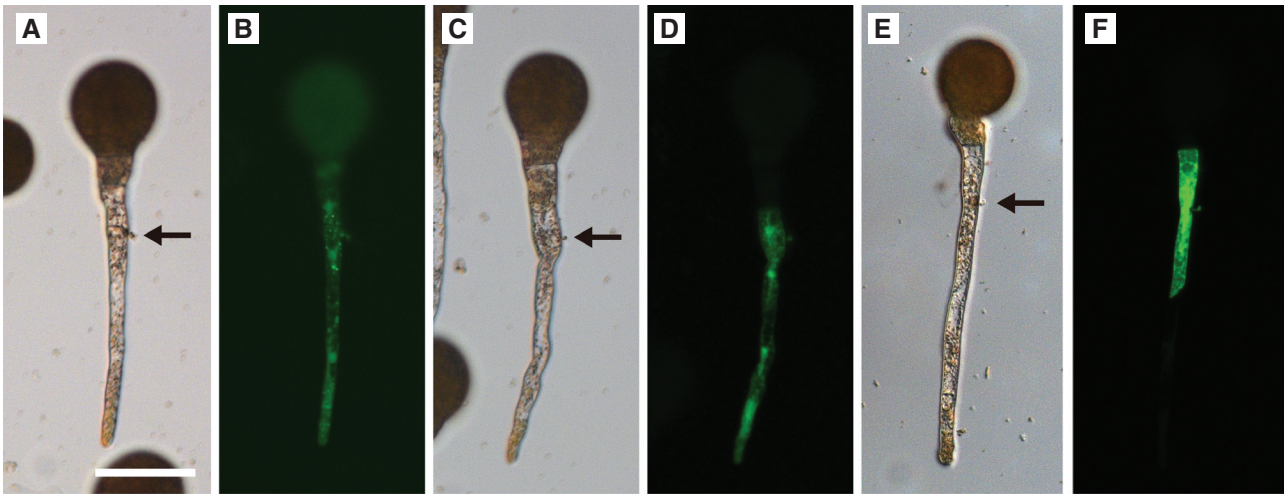

Figure 6: Microinjection of fluorescent tracers into sporophytes of *Fucus distichus* 10 days after fertilization. (A, C, E) Differential interference contrast microscope images of injected sporophytes. Arrows show the injected cells (B, D, F). Fluorescence microscope images of same sporophytes as in (A), (C), and (E). (A, B) Sporophyte injected with 3 kDa FITC-dextran. (C, D) Sporophyte injected with 10 kDa FITC-dextran. (E, F) Sporophyte injected with 40 kDa FITC-dextran. Scale bar=100 μm.

certain regions and periods. In the case of young sporophytes of *Fucus distichus*, *de novo* formation of secondary plasmodesmata was always observed in the first cell division plane. This implies that they have the potential to be a suitable model for studying the generation of secondary plasmodesmata.

In land plants, the difference in function between primary and secondary plasmodesmata has been discussed recently (Burch-Smith et al. 2011). The SEL in land plants is 1–3 kDa (Erwee and Goodwin 1983, Goodwin 1983, Terry and Robards 1987, Kempers et al. 1993, Radford and White 2001, 2011). Genetic analysis of plasmodesmata showed that a high occurrence of branched plasmodesmata is related to the ability for transportation of larger molecules compared with the wild type (Kim et al. 2002, Burch-Smith and Zambryski 2010, 2012). Another report shows that branched plasmodesmata have different properties (Oparka et al. 1999). Expressed green fluorescent protein (GFP)-fusion proteins (50 kDa) freely migrate to the sink leaf region through simple plasmodesmata. In the sink-source transition zone of leaves (a zone of physical change from carbon import to carbon export), the movement of the GFP-fusion proteins was limited, and the proportion of branched plasmodesmata increased compared with the simple plasmodesmata in the sink region. Oparka et al. (1999) suggested that the branched plasmodesmata work as "molecular sieves" to prevent nonspecific transportation within the leaf. On the other hand, we do not have observations about differences in function between the primary and second plasmodesmata of brown algae. As the first step to resolve this problem, the factors causing the formation of secondary plasmodesmata in brown algae should be examined.

The process of formation of secondary plasmodesmata is not well understood in land plants. Formation of secondary plasmodesmata requires several steps (Burch-Smith and Zambryski 2012): cell wall loosening and expansion, protrusion of plasma membrane in the loosened cell wall, break and perforation of the cell wall by protruded plasma membrane, fusion of plasma membrane between the neighboring cells via small pores in the cell wall. Finally, with the protrusion of the plasmodesmata, the tubular ER gets into the membrane canal in the plasma membrane. Cell wall loosening for the growth of land plants is caused by the actions of expansins, xyloglucan endotransglucosylase, and β-1,4-endoglucanase (Cosgrove 2005). The genome of *Ectocarpus siliculosus* does not encode for expansins and xyloglucan endotransglucosylase, and the cellulase family involved in the expansion of the cell wall in land plants (Cock et al. 2010, Michel et al. 2010). At present, there are no candidate proteins for cell wall loosening in brown algae. Cell wall loosening would be also an important step for the formation of secondary plasmodesmata in brown algae. It is necessary to examine how the cell wall is perforated, and plasma membrane penetrates into it to complete the plasmodesmata in brown algae.

The main role of plasmodesmata is to provide a route for symplastic cell-to-cell communication. In some species of Laminariales, translocation was examined using radioisotopes (Schmitz and Lobban 1976, Amat and Srivastava 1985). Long distance transport of ^{14}C-labeled

photosynthetic products demonstrated that brown algae have intercellular transportation systems. However, the mechanism that limits the size of molecules that can pass through the plasmodesmata remains unresolved. Only one earlier study has mentioned symplastic communication via plasmodesmata in brown algae (Bouget et al. 1998). Microinjection with FITC dextran (10 kDa) was performed in 3-, 8- and 12-celled zygotes of *Fucus spiralis*, and the tracer dispersed through all zygote cells. In the present study of *F. distichus*, 3 kDa FITC-dextran was dispersed through all the cells of the germlings. However, the movement of 10 kDa FITC-dextran was limited to the rhizoid region. These differences between *F. spiralis* and *F. distichus* may have been caused by the different developmental stages used for the microinjection test. We examined the movements of fluorescent tracers in young sporophytes that developed 10 days after fertilization. They were composed of more than 12 cells and already had recognizable cellular differentiation of the thallus, whereas the *F. spiralis* zygotes used for analysis by Bouget et al. (1998) would be undifferentiated. Pit fields were also observed in the thalli of *F. distichus*, which indicates that plasmodesmata in the pit fields could control the transport of larger molecules, in comparison with sparsely distributed plasmodesmata. Moreover, one reason for differences in SEL could be the aperture of the cell wall pores around the plasmodesmata. The diameter of the plasmodesmata was 10–20 nm, but the size of the cell wall pores around them in the thallus was smaller than in the rhizoid. If the transport potential of plasmodesmata is expanded by the surrounding cell wall pores, it is possible to explain why the plasmodesmata in the rhizoid have the ability to transport larger molecules. It is also possible that the inner structure of the plasmodesmata differs between thallus and rhizoid, so that the SEL would be affected. In the plasmodesmata of *Dictyota dichotoma*, internal bridges extending from plasma membrane were observed (Terauchi et al. 2012). This structure has not been confirmed in other brown algae, and the function remains obscure. The inner bridges could be involved in the transport function of plasmodesmata. Further cytological and molecular studies are needed to clarify the relationship between the distribution of plasmodesmata and the SEL in brown algae.

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References

Abe, M. 1970. A method of inducing egg liberation in *Fucus evanescens*. *Bot. Mag. Tokyo 83*: 254–255.

Amat, M.A. and L.M. Srivastava. 1985. Translocation of iodine in *Laminaria saccharina* (Phaeophyta). *J. Phycol. 21*: 330–333.

Bisalputra, T. 1966. Electron microscopic study of the protoplasmic continuity in certain brown algae. *Can. J. Bot. 44*: 89–93.

Bouget, F.Y., F. Berger and C. Brownlee. 1998. Position dependent control of cell fate in the *Fucus* embryo: role of intercellular communication. *Development 125*: 1999–2008.

Burch-Smith, T.M. and P.C. Zambryski. 2010. Loss of Increased Size Exclusion Limit (ISE)1 or ISE2 increases the formation of secondary plasmodesmata. *Curr. Biol. 20*: 989–993.

Burch-Smith, T.M. and P.C. Zambryski. 2012. Plasmodesmata paradigm shift: regulation from without versus within. *Annu. Rev. Plant Biol. 63*: 239–260.

Burch-Smith, T.M., S. Stonebloom, M. Xu and P.C. Zambryski. 2011. Plasmodesmata during development: re-examination of the importance of primary, secondary, and branched plasmodesmata structure versus function. *Protoplasma 248*: 61–74.

Cock, J.M., L. Sterck, P. Rouzé, D. Scornet, A.E. Allen, G. Amoutzias, V. Anthouard, F. Artiguenave, J.M. Aury, J.H. Badger, B. Beszteri, K. Billiau, E. Bonnet, J.H. Bothwell, C. Bowler, C. Boyen, C. Brownlee, C.J. Carrano, B. Charrier, G.Y. Cho, S.M. Coelho, J. Collén, E. Corre, C. Da Silva, L. Delage, N. Delaroque, S.M. Dittami, S. Doulbeau, M. Elias, G. Farnham, C.M. Gachon, B. Gschloessl, S. Heesch, K. Jabbari, C. Jubin, H. Kawai, K. Kimura, B. Kloareg, F.C. Küpper, D. Lang, A. Le Bail, C. Leblanc, P. Lerouge, M. Lohr, P.J. Lopez, C. Martens, F. Maumus, G. Michel, D. Miranda-Saavedra, J. Morales, H. Moreau, T. Motomura, C. Nagasato, C.A. Napoli, D.R. Nelson, P. Nyvall-Collén, A.F. Peters, C. Pommier, P. Potin, J. Poulain, H. Quesneville, B. Read, S.A. Rensing, A. Ritter, S. Rousvoal, M. Samanta, G. Samson, D.C. Schroeder, B. Ségurens, M. Strittmatter, T. Tonon, J.W. Tregear, K. Valentin, P. von Dassow, T. Yamagishi, Y. Van de Peer and P. Wincker. 2010. The *Ectocarpus* genome and the independent evolution of multicellularity in the brown algae. *Nature 465*: 617–621.

Cosgrove, D.J. 2005. Growth of the plant cell wall. *Nat. Rev. Mol. Cell. Biol. 6*: 850–861.

Ehlers, K. and R. Kollmann. 2001. Primary and secondary plasmodesmata: structure, origin, and functioning. *Protoplasma 216*: 1–30.

Erwee, M.G. and P.B. Goodwin. 1983. Characterisation of the *Egeria densa* Planch. leaf symplast. Inhibition of the intercellular movement of fluorescent probes by group II ion. *Planta 158*: 320–328.

Farnham, G., M. Strittmatter, S. Coelho, J.M. Cock and C. Brownlee. 2013. Gene silencing in *Fucus* embryos: developmental consequences of RNAi-mediated cytoskeletal disruption. *J. Phycol. 49*: 819–829.

Faulkner, C., O.E. Akman, K. Bell, C, Jefree and K. Oparka. 2008. Peeking into pit fields: multiple twinning model of secondary plasmodesmata formation. *Plant Cell 20*: 1504–1508.

Goodwin, P.B. 1983. Molecular size limit for movement in the symplast of the *Elodea* leaf. *Planta 157*: 124–130.
Hepler, P.K. 1982. Endoplasmic reticulum in the formation of the cell plate and plasmodesmata. *Protoplasma 111*: 121–123.
Kempers, R., D.A.M. Prior, A.J.E. Van Bel and K.J. Oparka. 1993. Plasmodesmata between sieve element and companion cell of extrafascicular stem phloem of *Cucurbita maxima* permit passage of 3 kDa fluorescent probes. *Plant J. 4*: 567–575.
Kim, I., F.D. Hempel, K. Sha, J. Pfluger and P.C. Zambryski. 2002. Identification of a developmental transition in plasmodesmatal function during embryogenesis in *Arabidopsis thaliana*. *Development 129*: 1261–1272.
Kim, J.-Y., Y. Rim, H. Wang and D. Jackson. 2005. A novel cell-to-cell trafficking assay indicates that the KNOX homeodomain is necessary and sufficient for intercellular protein and mRNA trafficking. *Genes Dev. 19*: 788–793.
Lucas, W.J., S. Bouché-Pillon, D.P. Jackson, L. Nguyen, L. Baker, B. Ding and S. Hake. 1995. Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. *Science 270*: 1980–1983.
McLean, B.G., F.D. Hempel and P.C. Zambryski. 1997. Plant intercellular communication via plasmodesmata. *Plant Cell 9*: 1043–1054.
Michel, G., T. Tonon, D. Scornet, J.M. Cock and B. Kloareg. 2010. The cell wall polysaccharide metabolism of the brown alga *Ectocarpus siliculosus*. Insights into the evolution of extracellular matrix polysaccharides in Eukaryotes. *New Phytol. 188*: 82–97.
Motomura, T. 1994. Electron and immunofluorescence microscopy on the fertilization of *Fucus distichus* (Fucales, Phaeophyceae). *Protoplasma 178*: 97–110.
Nagasato, C. and T. Motomura. 2002. Ultrastructural study on mitosis and cytokinesis in *Scytosiphon lomentaria* zygotes (Scytosiphonales, Phaeophyceae) by freeze-substitution. *Protoplasma 219*: 140–149.
Nagasato, C., A. Inoue, M. Mizuno, K. Kanazawa, T. Ojima, K. Okuda and T. Motomura. 2010. Membrane fusion process and assembly of cell wall during cytokinesis in the brown alga, *Silvetia babingtonii* (Fucales, Phaeophyceae) *Planta 232*: 287–298.
Nagasato, C., N. Kajimura, M. Terauchi, Y. Mineyuki and T. Motomura. 2014. Electron tomographic analysis of cytokinesis in the brown alga *Silvetia babingtonii* (Fucales, Phaeophyceae). *Protoplasma 251*: 1347–1357.
Oparka, K.J, A.G. Roberts, P. Boevink, S. Santa Cruz, I.M. Roberts, K.S. Pradel, A. Imlau, G. Kotlizky, N. Sauer and B. Epel. 1999. Simple, but not branched, plasmodesmata allow the nonspecific trafficking of proteins in developing tobacco leaves. *Cell 97*: 743–754.
Provasoli, L. 1968. Media and prospects for the cultivation of marine algae. *In*: (A. Watanabe and A. Hattori, eds) *Cultures and collections of algae*. Proc. US Japan Conf. Hakone, Jpn. Soc. Plant Physiol., pp. 63–75.
Radford, J.E. and R.G White. 2001. Effects of tissue-preparation-induced callose synthesis on est plasmodesma size exclusion limits. *Protoplasma 216*: 47–55.
Radford, J.E. and R.G White. 2011. Inhibitors of myosin, but not actin, alter transport through *Tradescantia* plasmodesmata. *Protoplasma 248*: 205–206.
Raven, J.A. 1997. Miniview: multiple origins of plasmodesmata. *Eur. J. Phycol. 32*: 95–101.
Robards, A.W. and W.J. Lucas. 1990. Plasmodesmata. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 41*: 369–419.
Schmitz, K. and C.S. Lobban. 1976. A survey of translocation in Laminariales (Phaeophyceae). *Mar. Biol. 36*: 207–216.
Terauchi, M., C. Nagasato, N. Kajimura, Y. Mineyuki, K. Okudai, C. Katsaros and T. Motomura. 2012. Ultrastructural study of plasmodesmata in the brown alga *Dictyota dichotoma* (Dictyotales, Phaeophyceae). *Planta 236*: 1013–1026.
Terauchi, M., C. Nagasato and T. Motomura. 2015. Plasmodesmata of brown algae. *J. Plant Res. 128*: 7–15.
Terry, B.R. and A.W. Robards. 1987. Hydrodynamic radius alone governs the mobility of molecules through plasmodesmata. *Planta 171*: 145–157.
Wakana, I. and M. Abe. 1992. Artificial insemination 'regulated by EDTA' in the monoecious brown alga *Fucus evanescens*. *Plant Cell Physiol. 33*: 569–575.
Zavaliev, R., S. Ueki, B.L. Epel and V. Citovsky. 2011. Biology of callose (β-1, 3-glucan) turnover at plasmodesmata. *Protoplasma 248*: 117–130.
Zavaliev, R., A. Levy, A. Gera and B.L. Epel. 2013. Subcellular dynamics and role of *Arabidopsis* β-1,3-glucanases in cell-to-cell movement of tobamoviruses. *Mol. Plant Micr. Inter. 26*: 1016–1030.

Bionotes

Chikako Nagasato
Muroran Marine Station, Field Science Center for Northern Biosphere, Hokkaido University, Muroran 051-0013, Japan,
nagasato@fsc.hokudai.ac.jp

Chikako Nagasato obtained her PhD from Hokkaido University, Japan. She has been studying mitosis and cytokinesis of brown algae.

Makoto Terauchi
Research Center for Inland Seas, Kobe University, Kobe 657-8501, Japan

Makoto Terauchi obtained his PhD from Hokkaido University, Japan, in 2014. He works as a post-doc researcher at Kobe University. He studies structural property of cell wall in brown algae.

Atsuko Tanaka
Muroran Marine Station, Field Science Center for Northern Biosphere, Hokkaido University, Muroran 051-0013, Japan

Atsuko Tanaka obtained her PhD from Kobe University, Japan. After 3 years as a post-doc researcher in France, she joined Muroran Marine Station, Hokkaido University, in 2013. Her current research interest is tissue organization of brown algae.

Taizo Motomura
Muroran Marine Station, Field Science Center for Northern Biosphere, Hokkaido University, Muroran 051-0013, Japan

Taizo Motomura obtained his PhD from Hokkaido University, Japan. His field of interest is morphology and developmental biology in macroalgae.