Plasmodesmata of brown algae

Makoto Terauchi · Chikako Nagasato · Taizo Motomura

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Abstract Plasmodesmata (PD) are intercellular connections in plants which play roles in various developmental processes. They are also found in brown algae, a group of eukaryotes possessing complex multicellularity, as well as green plants. Recently, we conducted an ultrastructural study of PD in several species of brown algae. PD in brown algae are commonly straight plasma membrane-lined channels with a diameter of 10–20 nm and they lack desmotubule in contrast to green plants. Moreover, branched PD could not be observed in brown algae. In the brown alga, *Dictyota dichotoma*, PD are produced during cytokinesis through the formation of their precursor structures (pre-plasmodesmata, PPD). Clustering of PD in a structure termed “pit field” was recognized in several species having a complex multicellular thallus structure but not in those having uniseriate filamentous or multiseriate one. The pit fields might control cell-to-cell communication and contribute to the establishment of the complex multicellular thallus. In this review, we discuss fundamental morphological aspects of brown algal PD and present questions that remain open.

Keywords Brown algae · Multicellularity · Pit field · Plasmodesmata · Primary plasmodesmata · Secondary plasmodesmata

Introduction

Brown algae are multicellular photosynthetic eukaryotes that are found in marine environments. They include species of various sizes from microscopic to large exceeding tens of meters, namely, giant kelps. In many cases, brown algae have complex life cycles that include sexual dimorphism, and the alternation occurs between the sporophyte and gametophyte generations (Luthringer et al. 2014; Silberfeld et al. 2010; Wynne and Loiseaux 1976). The two generations are connected by asexual and sexual reproductions with diverse modes. Brown algae have evolved unique systems such as a complex life cycle, during adaptation to coastal environments.

Brown algae, together with diatoms, belong to the heterokontophyta and to the phylum of “Stramenopiles” with other heterotrophic organisms including Oomycetes. Stramenopiles are phylogenetically distant from the Opisthokonta (animals and fungi) and the Archaeplastida (green plants and red algae) (Yoon et al. 2004). Brown algae evolved complex multicellularity independently of animals, fungi, green plants and red algae. These five multicellular groups have developed intercellular connections, namely gap junctions in animals (Caspar et al. 1977; Hervé and Derangeon 2013; Kumar and Gilula 1996), septal pores in fungi (Bauer et al. 2006; Marchant 1976; Reichle and Alexander 1965), pit plugs in red algae (Pueschel 1977; Pueschel and Cole 1982) and plasmodesmata (PD) in green plants (Burch-Smith et al. 2011) and brown algae (Schmitz and Srivastava 1974; Schmitz 1981, 1990; Terauchi et al.

M. Terauchi
Graduate School of Environmental Science, Hokkaido University,
Sapporo 060-0810, Japan
e-mail: terauchi1117@people.kobe-u.ac.jp

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

C. Nagasato (✉) · T. Motomura
Muroran Marine Station, Field Science Center for Northern
Biosphere, Hokkaido University, Muroran 051-0003, Japan
e-mail: nagasato@fsc.hokudai.ac.jp

2012). Intercellular connections allow cell-to-cell communication through the transport of various molecules and contribute to the elaboration of the complex multicellularity (Bloemendal and Kück 2013).

In land plants, PD are plasma membrane-lined tubular channels with a diameter of 30–50 nm, creating symplastic continuity across the cell wall. Endoplasmic reticulum (ER, desmotubule), characteristically passes through the PD lumen. PD of land plants are categorized into two types: unbranched “simple PD” and branched “complex PD”. Molecules transported via PD include ions, small compounds, proteins and RNA (Kim 2005). Transport of these materials via PD is highly regulated and is involved in a number of developmental processes in land plants (Burch-Smith et al. 2011). The structure of PD is much different from that of gap junctions of animals; these 2–4 nm wide proteinous channels facilitate the transport of small molecules up to about 1 kDa such as ions, secondary signaling messengers, nucleotides and metabolites (Hervé and Derangeon 2013; Maeda and Tsukihara 2011). Septal pores of fungi are 50–500 nm wide plasma membrane-lined pores co-localized with peroxisome-derived vesicles or ER-derived septal pore caps. They contribute to the cellular differentiation (Bauer et al. 2006; Reichle and Alexander 1965; van Peer et al. 2010). Pit plugs of red algae consist of a proteinaceous plug core occluding a pore lined by plasma membrane in the cell wall and cap membrane covering both sides of the plug core (Pueschel and Cole 1982). The structure of pit plug provides significant taxonomical information (Pueschel and Cole 1982).

In brown algae, studies on intercellular transport have focused on sieve elements in kelps. In some laminariales, the differentiation of tissues consisting of epidermal, cortex and medullary cells is conspicuous, and medullary cells (sieve elements) are functionally analogous to those of land plants. The sieve elements of brown algae are continuous to cortex cells via the complex filamentous cell network (Schmitz 1984). The monitoring of transport of isotopes (^{14}C , ^{32}P , ^{125}I) showed that the long-distance transport of photosynthetic products and iodine occurred through the sieve elements (Amat and Srivastava 1985; Schmitz and Srivastava 1975, 1979). The cross walls of sieve elements are perforated by numerous pores, linking adjacent sieve elements. The diameter of the pores ranges from 37.5 nm to 2.6 μm (Schmitz 1990). Although smaller pores can be regarded as PD, larger pores are predicted to be specialized structures of PD that are formed by the enzymatic digestion of the cross wall (Schmitz and Srivastava 1974; Marchant 1976; Schmitz 1981, 1990). The detail of the structure and the function of PD in other cell types and algal species remain obscure.

Considering the distant evolutionary relationship between brown algae and green plants, they must have

evolved PD independently (Raven 2008). The similarity of molecular components between brown algal PD and those of green plants might be low (Cock et al. 2010; Salmon and Bayer 2013). Structural and functional analyses of PD will give insights into how brown algae established independently complex multicellularity. Recently, we carried out ultrastructural observations of PD in the brown alga *Dictyota dichotoma* (Terauchi et al. 2012). We characterized their detail structure and formation during cytokinesis. In this review, we summarize our current knowledge on the structure of brown algal PD and compare them with green plants.

Ultrastructure of brown algal PD and its relationship to the molecular traffic

All brown algae are multicellular species organized in branching uniseriate and multiserial filamentous, and complex multicellular thalli. For example, *Dictyota dichotoma* forms a macroscopic complex multiserial thallus (Fig. 1a), *Sphacelaria rigidula* forms a filamentous multiserial thallus (Fig. 1b), and *Ectocarpus siliculosus* forms a filamentous uniseriate thallus (Fig. 1c). Transmission electron microscopic (TEM) observations showed that vegetative cells of all species examined had ER (desmotubule)-free PD with an inner diameter ranging from 10 to 20 nm and a length from 1 to several 100 nm (Fig. 1d–f). Branched complex PD were never observed in brown algae, in contrast to land plants. Although in one of the published figures of pores in the sieve element from *Laminaria groenlandica* (Fig. 18 of Schmitz and Srivastava 1974) it has been reported that they contain ER, the existence of desmotubule in brown algal PD has never been described elsewhere. The occurrence of complex PD and desmotubule has been described in some members of bryophytes and charophycean algae (Cook et al. 1997; Franceschi et al. 1994) but not in other green algae (Fraser and Gunning 1969). It was argued that those specializations of PD occurred during the evolution toward land plants in the green lineage (Cook et al. 1997). Ultrastructural observations of brown algal PD in vegetative cells showed that PD have similar form from simple uniseriate to complex multicellular species, while PD (diameter 10–20 nm) and pores of sieve elements (diameter 37.5 nm–2.6 μm) significantly differ in their diameter. In Fucales and Laminariales, the number and size of the pores are variable among species and among sieve elements from different parts or ages of the thallus, and even within one cross wall (Moss 1983; Schmitz and Srivastava 1974, 1976). Although structure may differ depending on tissue fixation method used (chemical fixation or cryofixation), regulation of the diameter of PD and pores may be the main determinant for molecular transport conductance in brown algae as well as

Fig. 1 General view of thallus and ultrastructure of PD in **a, d** *Dictyota dichotoma* (sporophyte, Dictyotales), a complex multicellular thallus; **b, e** *Sphacelaria rigidula* (male gametophyte, Sphacelariales), a multiseriate thallus; **c, f** *Ectocarpus siliculosus* (male sporophyte, Ectocarpales), a filamentous uniseriate thallus. TEM samples were prepared by rapid freezing/freeze substitution. Insets show the transverse views of plasmodesmata. ER-free PD penetrate the electron-dense cell wall (cw). Abbreviations: chl, chloroplast; cw, cell wall; pd, plasmodesma; pm, plasma membrane; py, pyrenoid. Scale bars: 20 μ m (**a–c**), 100 nm (**d–f**), 20 nm (inset of **d–f**)

in green plants. PD with a large diameter in the sieve element can be regarded as pores specialized for the long-distance transport. In land plants, it has been reported that PD determine the upper limit of molecular weight of the cargo macromolecules (size exclusion limit, SEL) (Christensen et al. 2009; Zambryski 2004). Degradation and synthesis of callose (β 1, 3-glucan) at the neck region of PD is one of the molecular mechanisms for controlling SEL; when the cell receives endogenous (e.g. developmental process) and exogenous (e.g. pathogen infection and cell injury) signals, callose is deposited at the neck region of PD, resulting in a decrease of the diameter of PD and SEL, while callose degradation reverses the effect (Zavaliev et al. 2011). SEL is varied in species, tissues, and developmental stages. In a study of *Elodea canadensis* leaf cells microinjected with fluorescent-labeled peptides, SEL was estimated to be less than 1 kDa (Goodwin 1983). In the analysis of tobacco leaf cells expressing a green fluorescent protein (GFP, fusion protein), SEL was estimated to be around 50 kDa in sink leaves while greatly reduced in source leaves (Oparka et al. 1999). In *Zea mays*, microinjection of GFP and fluorescent-labeled dextran in coleoptile epidermal cells showed that the intercellular movement of the dextran probe (4.4 kDa) was limited and movement of GFP was also observed to some extent (Wymer et al. 2001). SEL in these cells was predicted to be around 4.4 kDa. Theoretically, globular proteins with an approximate diameter of 9 nm correspond to a molecular weight of 45 kDa (Lucas and Wolf 1993),

which predicts that the macromolecular transport via PD in brown algae may be possible in terms of their diameter (i.e. 10–20 nm). FITC-dextran (10 kDa) microinjected in early developmental stage zygotes of the brown alga *Fucus spiralis* was transported throughout the young sporophyte, suggesting that PD provides the functional symplastic route for the intercellular transport of molecules in brown algae (Bouget et al. 1998). However, it is yet unclear whether the temporal or permanent alteration of diameter of PD regulates SEL in brown algae.

Formation of brown algal PD

In land plants, two types of PD exist: the primary PD formed during cytokinesis and the secondary PD which have a post-cytokinetic origin. Primary PD are generated by the physical obstruction of ER incorporated into the cell plate as shown by TEM observations (Hepler 1982). Secondary PD are synthesized *de novo* by the local degradation of the cell wall and protrusion of plasma membrane and desmotubule or by addition of branches to primary PD (Lucas and Wolf 1993; Faulkner et al. 2008). In several brown algae, PD-like structures were observed in the cell partition membrane during cytokinesis (La Claire 1981; Katsaros et al. 2009). Recently, we confirmed by electron tomographic analysis that the precursor structures of brown algal PD, pre-plasmodesmata (PPD), occurred during cytokinesis in *D. dichotoma* (Terauchi et al. 2012). In

Fig. 2 Formation of PD during cytokinesis in *D. dichotoma*. **a** Electron micrograph of cytokinesis of a meristematic epidermal cell. Cytokinesis proceeds by expansion of the membranous sacs arranged on the cytokinetic plane as patches (arrowheads). **b** Longitudinal view of pre-plasmodesmata (PPD) in the developing cell partition membrane. Arrowhead indicates one PPD. **c** Transverse view of PPD in the developing cell partition membrane. Arrowhead indicates one PPD. Abbreviations: chl, chloroplast; n, nucleus; pm, plasma membrane. Scale bars: 500 nm (a), 100 nm (b), 200 nm (c)

cytokinesis of brown algae, Golgi vesicles and flat cisternae take part in the formation of the cell partition membrane (Nagasato and Motomura 2002, 2009; Katsaros et al. 2009; Nagasato et al. 2010, 2014). Similarly, in *D. dichotoma*, cytokinesis proceeds by the expansion of patches of membranous sacs which are formed by the fusion of Golgi vesicles and flat cisternae (Fig. 2a). In the developing cell partition membrane, tubular membranous structures (PPD) are recognized (Fig. 2b, c). It was suggested that PPD derived from the invagination of the membranous sac in *D. dichotoma* (Fig. 3a). Their inner diameter ranges from 10 to 20 nm as in mature PD. They are evenly distributed in specific areas of the membranous sacs and persist after completion of the cell partition membrane (Fig. 3b). Mature PD are clustered in a part of the cell walls called the “pit fields”, cluster of PD corresponding to the deeper side of epidermal cells near the underlying medullary cells (see next paragraph for a detail description of the pit fields). PPD are preferentially formed in the restricted region of the cell wall where the mature PD are formed. This indicates

Fig. 3 Schematic illustration of the formation of PD in *D. dichotoma* (drawn from Terauchi et al. 2012). **a** Early stage of cytokinesis. Golgi vesicles and flat cisternae (not shown) fuse to form the membranous sac (MS). Cytokinesis proceeds by the expansion of MS (arrows). PPD formation begins in the restricted region of MS by the protrusion (arrowheads) of MS membrane from either side of MS. **b** Late stage of cytokinesis before the initial cell wall development. The cell partition membrane contains PPD-rich region (PR). **c** Mature cell wall. Plasmodesmata (PD) are clustered in the thin cell wall (CW) region as a pit field, and then the PD-free cell wall gets thickened

that the PPD distribution in the newly forming cell partition membrane matches the sites of the “future” pit field (Fig. 3c). These data confirm that brown algae have primary PD that are produced in a manner different from land plants. It is unclear how PPD are constructed at the molecular level and how their position in the cell partition membrane is determined.

Are secondary PD present in brown algae? In previous studies of the first and second cytokinesis of zygotes of *Scytosiphon lomentaria* (Nagasato and Motomura 2002) and *Silvetia babingtonii* (Nagasato et al. 2010, 2014), PPD were not observed in the first and second cell partition membrane. In the mature thalli of *S. lomentaria* and *S. babingtonii*, we observed dense PD in the cell wall.

Table 1 Comparison of PD distribution in cell wall in several species of brown algae

Organism	Pit field ^a	Area of pit field (μm^2)		No. pit field per wall	PD frequency (μm^{-2})		No. PD per pit field	Distance between PD (nm)	
		mean $\pm$ SD	n		mean $\pm$ SD	n		mean $\pm$ SD	n
Sphacelariales	—							265 $\pm$ 151	5
<i>Sphacelaria rigidula</i> (male gametophyte)									
Ectocarpales	—				13 $\pm$ 5	6		251 $\pm$ 131	132
<i>Ectocarpus siliculosus</i> (male sporophyte)									
Scytosiphonales	+	1.1 $\pm$ 0.6	4	Unknown	73 $\pm$ 5 ^b	4	80 ^e	97 $\pm$ 16	15
<i>Scytosiphon lomentaria</i> (gametophyte)									
Laminariales	+	0.3 $\pm$ 0.2	3	Multiple	226 $\pm$ 41 ^c	3	68	71 $\pm$ 13	112
<i>Saccharina japonica</i> (sporophyte)									
Desmarestiales	+	4.4 $\pm$ 1.5	2	Unknown	51 $\pm$ 3	3	224	118 $\pm$ 32	87
<i>Desmarestia ligulata</i> (sporophyte)									
Fucales	+	9.8 $\pm$ 5.8	3	1	90 $\pm$ 10	7	882	81 $\pm$ 12	72
<i>Fucus distichus</i> (sporophyte)									
Dictyotales	+	0.6 $\pm$ 0.1	3	Multiple	332 $\pm$ 38 ^d	3	199	62 $\pm$ 16	226
<i>Dictyota dichotoma</i> (sporophyte)									
PPD before initial cell wall development								81 $\pm$ 17	265
PPD after initial cell wall development								74 $\pm$ 11	62

PD frequency per $1 \mu\text{m}^2$ was calculated using the absolute frequency

^a + pit fields observed — no pit field observed

^b The measured areas were smaller than $1 \mu\text{m}^2$. The absolute frequency is expressed as the number of (no.) PD per $0.5 \mu\text{m} \times 0.5 \mu\text{m}$

^c The measured areas were smaller than $1 \mu\text{m}^2$. The absolute frequency is expressed as no. PD per $0.4 \mu\text{m} \times 0.4 \mu\text{m}$ or $0.25 \mu\text{m} \times 0.25 \mu\text{m}$

^d The measured areas were smaller than $1 \mu\text{m}^2$. The absolute frequency is expressed as no. PD per $0.5 \mu\text{m} \times 0.5 \mu\text{m}$ or $0.25 \mu\text{m} \times 0.25 \mu\text{m}$

^e Calculated using mean area of the pit field (μm^2) and mean PD frequency (No. PD μm^{-2})

Additionally, at later stages of *S. babingtonii* zygote development, PD are present in the newly formed cell wall (unpublished data). There is a high possibility that brown algae have the capability to generate secondary PD.

PD distribution and their implication for the body plan in brown algae

Frequency and distribution of PD in the cell wall should play important roles in cell-to-cell communication in brown algae. In *D. dichotoma*, PD are clustered at the pit fields and localized in the thin cell wall. Clustering appears during cytokinesis (Terauchi et al. 2012). In our study, the pit fields were observed in many species examined (Dictyotales, Laminariales, Fucales, Desmarestiales, Scytosiphonales) (Table 1; Figs. 4, 5). The pit fields were present in the thin cell wall in Dictyotales, Laminariales and Fucales and in the relatively uniform cell wall in other species (Desmarestiales and Scytosiphonales). The pit fields are round or oval shaped and their number, mean area

and PD frequency in the cell wall varies between species (Table 1; Fig. 4a, b). The pit fields could not be identified in Sphacelariales species (Fig. 4c) and *E. siliculosus* (Fig. 4d). In these species, PD are dispersed over the cell wall and the distance between PD (distance between the center of one plasmodesma and that of adjacent one averages about 250 nm) was much longer than the distance between PD in the pit fields (average: 60–120 nm) (Table 1). The presence and absence of the pit fields was seen in different generations of the life cycle. *S. japonica* undergoes alternation between heteromorphic generations: macroscopic complex multicellular sporophyte and microscopic filamentous gametophyte. The thallus of well-developed sporophyte is composed of several layers of epidermal, cortex and medullary cells (Fig. 5a). PD traversed the cell wall making pit fields at places where cell wall thickness is reduced to about $0.1 \mu\text{m}$ (Fig. 5b). Tubular and other membranous structures in the cytoplasm are localized just beneath the pit field (arrowheads in Fig. 5b). Multiple round- or oval shaped-pit fields are present in the central part of the cell wall

Fig. 4 PD distribution in several brown algae. TEM samples were prepared by chemical fixation in **a, b**, and by rapid freezing/freeze substitution in **c, d**. **a** Transverse view of PD (pit field) between cortex cells in *Fucus distichus* (sporophyte, Fucales). Inset: overview of the cell wall. The pit field is located in the center of the cell wall (arrowhead). **b** Transverse view of PD between cortex cells in *Desmarestia ligulata* (sporophyte, Desmarestiales). Note that both species show the clustering of PD (pit field) but PD frequency is different. **c** Transverse view of PD in *Halopteris paniculata* (sporophyte, Sphacelariales). **d** Transverse view of PD in *E. siliculosus* (male sporophyte, Ectocarpales). Note that in both species PD are dispersed in the septum cell wall and their frequency is quite low (arrowheads). Scale bars: 2 μm (inset of **a**), 200 nm (**a, b**), 500 nm (**c, d**)

(Fig. 5c, arrowheads in Fig. 5d, e) or its periphery. Similar features of PD were reported in other laminarialean species (Schmitz and Kühn 1982). The gametophyte of *S. japonica* has a low PD frequency and lacks pit fields (Fig. 5f, g) although a few PD are often located close together (arrowheads in Fig. 5g). The PD frequency has been reported in the above study. In the cortex cells of *Saccharina latissima* (Laminaria saccharina), it is 100–200 PD μm^{-2} (Schmitz and Kühn 1982), and the authors calculated the PD frequency from published micrographs of other brown algae: 168 PD μm^{-2} in *Himantalia lorea* (Fucales), 132–172 PD μm^{-2} in *Egregia menziesii* (Laminariales). The PD frequency in *S. japonica* (Table 1) correlates well with the calculated PD frequencies. In cross walls of sieve elements, the pore frequency was 0.03–100 pores μm^{-2} in the Laminariales (Schmitz and Srivastava 1974, 1975, 1976; Schmitz 1981, 1990), 50–60 pores μm^{-2} in the Fucales and 2,000–3,000 pores μm^{-2} in *Dictyopteris membranacea* (Dictyotales, Katsaros and Galatis 1988). It was suggested that the pore frequency greatly depends on the pore diameter (Schmitz 1990). The distance between PD in the pit fields (Table 1) validates that they are arranged at almost

regular intervals. The even pore distribution was observed in the sieve element of *Fucus vesiculosus* (Fielding et al. 1987) and *D. membranacea* (Katsaros and Galatis 1988). In *D. dichotoma*, the distance between PPD in the newly formed cell partition membrane was 81 ± 17 nm before, and 74 ± 11 nm after the initial cell wall development (Table 1). This means that PPD are also arranged at almost regular intervals in the cell partition membrane similar to the case of the mature pit fields. The process of pit field formation in land plants is different from that of brown algae. In four plant species (*Trifolium repens*, *Raphanus sativus*, *Zea mays*, and *Sorghum vulgare*), comparison of PD distribution in root meristem cells and elongating cells, provides evidence that the clustering of PD and secondary PD formation take place during cell wall expansion (Seagull 1983). As a result of cell wall expansion, there is a general shift from dispersed to clustered PD and the PD frequency is maintained even after cell wall expansion due to secondary PD formation (Seagull 1983). Observation of PD in the basal cell walls of trichomes during leaf development in tobacco demonstrated that there is a shift from randomly distributed simple PD to the pit field containing many paired PD during cell wall expansion. Land plants possess a system that inserts secondary PD into the vicinity of primary PD giving rise to pit fields composed of complex PD (Faulkner et al. 2008). The pit fields of land plants have a post-cytokinetic origin and the arrangement of PD can be changed during cell wall expansion. The pit fields of brown algae and land plants are much different in 1) the timing and process of their formation, 2) the presence or absence of branched complex PD within the pit fields, 3) the arrangement of PD within the pit fields. Secondary PD in brown algae might be inserted around primary PD at regular intervals increasing the surface area of the pit fields.

There is no experimental data so far to explain how pit fields participate in the establishment of the complex multicellular system. One possibility might be that since the pit fields contain a number of PD (Table 1), the increase of the total number of PD per cell wall interface could lead to the higher flux rate of the molecular transport and active cell-to-cell communication. The PD frequency in the septum of filamentous gametophyte of *S. japonica* is quite low. While PD frequency is much lower in *E. siliculosus* than in other complex multicellular species, the total number of PD in the septum was estimated to be quite high. The area of the septum is about 300 μm^2 when the diameter of the cylindrical cell is 20 μm . Since PD of *E. siliculosus* are dispersed over the septum (average 13 PD μm^{-2}), the total number of PD in the septum will be about 4,000. This number is much higher than that of any other species examined. Therefore, the total number of PD between cells is not an absolute determinant of the complex multicellular thallus structure. The area, number and position

Fig. 5 PD of *Saccharina japonica* (Laminariales). **a** Overview of sporophyte thallus. TEM samples were prepared by chemical fixation. Small epidermal cells (*E*) are seen in the *upper side*. Cortex cells (*C*) in the *central part* are large and highly vacuolated cells. Medulla (*M*) with expanded extracellular matrix is visible in the *lower part*. **b** Longitudinal view of PD between cortex cells. No desmotubule can be seen in the lumen of the plasmodesma. Membranous structures are present in the vicinity of PD (arrowheads). **c, d** Transverse view of PD between cortex cells. The image in **d** is magnified from the dotted rectangle in **c**. Note that three pit fields are present in the central

part of the cell wall (arrowheads in **d**). **e** Transverse view of one pit field. Note that the distance between PD is almost constant. **f** Overview of male gametophyte thallus. TEM samples were prepared by rapid freezing/freeze substitution using culture strains. Gametophyte has uniseriate filamentous thallus. **g** Transverse view of PD. PD frequency is much lower than that of sporophyte and do not gather as the pit field but PD are often located near to each other (arrowheads). Abbreviations: *cw*, cell wall. Scale bars: 10 μm (**a, f**), 2 μm (**c, d**), 100 nm (**b, e, g**)

of pit fields may comprehensively influence the pattern of molecular transport. This idea is supported by the report in laminarialean species that those properties of the pit fields are different between anticlinal and periclinal cell walls of epidermal and cortex cells, which determines the transport pattern of photosynthetic products from epidermis toward medulla (sieve elements) (Schmitz and Srivastava 1975; Schmitz 1981; Schmitz and Kühn 1982). One hypothesis is that cargo molecules and components that mediate the traffic via PD are gathered into the pit fields, thereby allowing effective and synchronized regulation of the molecular flux rate, direction and selection of the cargo molecules through each plasmodesma. If this mechanism exists, it could achieve a more dynamic and strict regulation of intercellular molecular traffic via the pit fields than via dispersed PD.

Concluding remarks and future perspective

We have investigated the morphology of brown algal PD, but many aspects still remain unclear and require further investigation: (1) the correlation between the formation of the pit fields and establishment of the complex multicellular body plan needs further validation. (2) The molecules that are transported via PD need to be inventoried and (3) proteins that make up PD should be identified. Moreover, it is also important to establish whether the PD distribution in the cell wall is fixed during cytokinesis or flexibly adjusted during the developmental process. Brown algae probably have secondary PD that are added to the pre-existing pit fields or other cell walls. It is unknown whether *de novo* post-cytokinetic insertion of the pit fields into the PD-free

cell wall takes place in brown algae. In land plants, pre-existing PD can be removed from the cell wall during the developmental process. For example, it is well known that cell walls in guard cells lose PD and become symplastically isolated from surrounding cells during their maturation (Wille and Lucas 1984). It is not known whether the elimination of pre-existing PD occurs in brown algae. From the reports of the absence of PD in the first cell partition membrane of zygotes of several brown algae (Nagasato and Motomura 2002; Nagasato et al. 2010, 2014), we can infer that some vegetative cells of the developing thallus as well as zygotes might undergo PPD-free cytokinesis. However, cells of mature thalli of all species examined had PD. The complete symplastic isolation by the absence of PD may be rare in brown algae. In land plants, it was reported that the local grouping of cells by SEL of PD, called “symplastic field”, was the fundamental mechanism in creating the positional information, and achieving cell and tissue differentiation (Kim et al. 2004). In brown algae, the existence of a symplastic field is unknown. In the early developmental stage of zygotes in *D. dichotoma*, *F. disticus* and *S. japonica*, pit fields were not observed and the PD frequency was low (unpublished data). The onset of pit field formation might be regulated according to the developmental schedule. Brown algal PD still leaves many puzzles to be solved. The data presented here could serve as a framework to a detailed functional analyses of brown algal PD.

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