In: Cytoskeleton: Cell Movement... ISBN: 978-1-60876-559-1
Editors: S. Lansing et al., pp. 205-218 © 2010 Nova Science Publishers, Inc.

Chapter VII

Migrating Plant Cell: F-Actin Asymmetry Directed by Phosphoinositide Signaling

Koji Mikami*
Faculty of Fisheries Sciences, Hokkaido University,
3-1-1 Minato-cho, Hakodate 041-8611, Japan.

Abstract

Polarity is a fundamental cell property essential for differentiation, proliferation and morphogenesis in unicellular and multicellular organisms. It is well known that polarized distribution of F-actin is important in providing the driving force for directional migration in mammalian leukocytes and *Dictyostelium* cells. Phosphoinositide (PI) signaling, including phosphatidylinositol kinases and phospholipases, is also critical for the formation of cell polarity in these cells. A monospore from the marine red alga *Porphyra yezoensis* is well known as a migrating plant cell and thus is a unique and useful material for investigating polarity determination in plant cells. As in leukocytes and *Dictyostelium* cells, monospore migration requires asymmetrical distribution of F-actin, whose establishment is regulated by the phosphatidylinositol 3-kinase and phospholipase C, whereas

* Corresponding author: Tel/Fax: +81-138-40-8899; E-mail: komikami@fish.hokudai.ac.jp

phospholipase D is involved in the maintenance of F-actin distribution. These findings indicate that the regulation of F-actin asymmetry by PI signaling cascades is evolutionarily conserved in terms of the establishment of cell polarity in migrating eukaryotic cells.

Introduction

The initial establishment of cell polarity, exhibited as asymmetrical cell division and directional migration, depends on asymmetrical cues that lead to reorganization of the cytoskeleton and polarized distribution of cortical proteins and membrane lipids (Iglesias and Devreotes, 2008; Janetopoulos and Firtel, 2008). When *Dictyostelium* cells and leukocytes respond to external stimuli such as cAMP and cytokines, cells in the axialized form can rapidly change their body shape along with the formation of cell polarity in response to the chemoattractant, following the rapid formation of a leading edge on the side of the cell exposed to the highest concentration of the chemoattractant with a trailing edge appearing on the opposite side (Iglesias and Devreotes, 2008; Janetopoulos and Firtel, 2008). Formation of the leading edge occurs in parallel with the polarized localization of F-actin, whereas assembled myosin II is enriched at the trailing edge. Thus, the polarized distribution of cytoskeletal components provides the driving and contractile forces required for directional cell migration during chemotaxis. Involvement of the cytoskeleton in the establishment of cell polarity has also been reported in land plants (Staiger, 2000; Hepler *et al*., 2001; Smith, 2003). F-actin and microtubules (MTs) have also been shown to play important roles in the establishment of polarity during tip growth of pollen tubes and root hairs (Fu *et al*., 2001; Sieberer *et al*., 2005). These findings show that the polarized accumulation of cytoskeletal elements, particularly F-actin, is important for the establishment of cell polarity in both animals and land plants.

The marine red alga *Porphyra yezoensis* has been proposed as a model marine plant for physiological and genetic studies in seaweed because of its biological and economic importance (Saga and Kitade, 2002). The life cycle of *P. yezoensis* is characterized as a heteromorphic haplodiploid type, in which the haploid and diploid phases are large leafy gametophyte and microscopic filamentous sporophyte, conchocelis, respectively (Klinger, 1993; Coelho *et al*., 2007). In addition to the sexual life cycle, *P. yezoensis* can propagate asexually using monospores, which are produced in the marginal

region of gametophytic blades (see Figure 1). It is noteworthy that the monospore is an example of a moving plant cell (Pickett-Heaps *et al*., 2001; Ackland *et al*., 2007); therefore, these cells have been employed to elucidate the regulatory mechanisms in the establishment of cell polarity required for cell migration in plant cells (Li *et al*., 2008; Li *et al*., 2009). This chapter will highlight the involvement of asymmetrical distribution of F-actin and phosphoinositide signaling in the formation of cell polarity required for monospore migration.

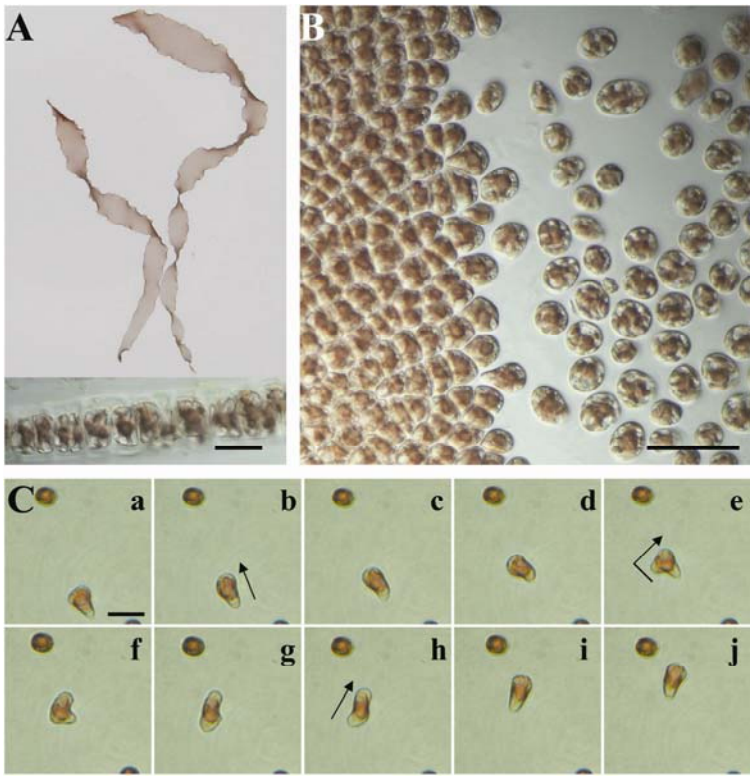

Figure 1. Discharge and movement of monospores from a gametophytic blade of *P. yezoensis*. (A) Leafy gametophyte of laboratory cultured *P. yezoensis* (strain TU-1); the image below shows a transverse section of a single layered gametophyte. Scale bar = 10 μm. (B) Release of monospores from the marginal region of a gametophyte. Scale bar = 50 μm. (C) Sequential images of monospore migration after release. Monospores usually showed directional migration (a-d, h-j); however, a change in the direction of polarized migration was sometimes observed (e -g). Eight minutes elapsed from panels a- j. Scale bar = 15 μm

Asymmetrical Distribution of F-Actin in Migrating Monospores

Gametophytes of *P. yezoensis* are flat sheets composed of one layer of cells (Figure 1A). From the edge of these flat sheets, monospores are released as round somatic cells (Figure 1B). Because of the lack of a flagellum, retractile and amoeboid migration of monospores is found in cells that have undergone morphological change, exhibiting a tapered tail after release (Figure 1C). Pharmacological studies have shown that motility of monospores was completely inhibited by the disruption of local F-actin accumulation by Cyt B and Lat B. This indicates that generating the force for directed migration is dependent on the organization of F-actin in monospores (Li *et al*., 2008). These findings are similar to those in the red alga *P. pulchella* (Ackland *et al*., 2007).

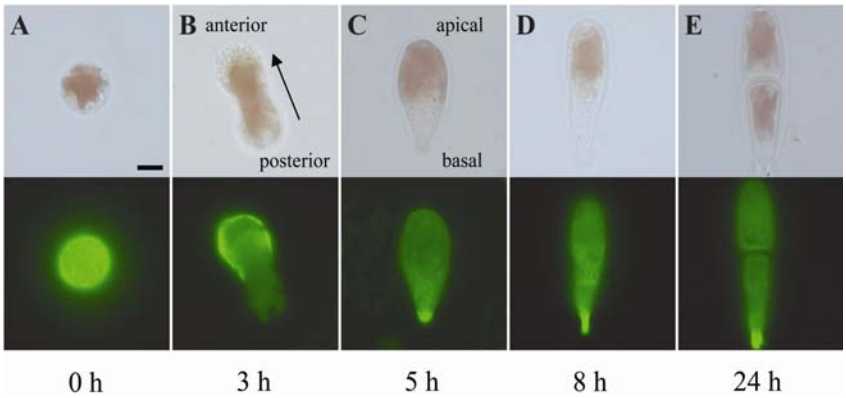

Figure 2. F-actin distribution during the early development of monospores. F-actin was stained with Alex Flour 488 phalloidin. Upper and lower photos in each panel show brightfield and fluorescent images, respectively. The times below the panels indicate the time elapsed after monospore release. (A) Newly released monospore. (B) Migrating monospore with the arrow indicating the direction of migration. (C) Adhering monospore. (D) Elongating monospore. (E) First asymmetric cell division. Scale bar = 5 μm.

Staining the cells with Alex Flour 488 phalloidin provided evidence of the clear relationship between F-actin accumulation and cell movement in monospores (Li *et al*., 2008; Li *et al*., 2009). In freshly released monospores,

actin filaments were observed as bundles in the cell (Figure 2A); however, once the monospores moved, F-actin became densely assembled at the leading edge (Figure 2B). Since F-actin is generally involved in cytoskeleton arrangement, which is crucial for the establishment and maintenance of cell polarity (Samaj *et al*., 2000; Jedd and Chua, 2002), localized accumulation of F-actin at the leading edge during movement is thought to play roles in the establishment and/or maintenance of cell polarity in monospores.

Asymmetrical localization of F-actin in monospores was also observed after conversion of cell axis from anterior-posterior to apical-basal during early development (Li *et al*., 2008). Monospores observed five hours after release were seen to adhere to the substratum, establishing the apical-basal axis and becoming upright by elongation of the bottom part of the cell (Figures 1C and D). After elongation, the first asymmetric cell division occurred perpendicular to the apical-basal axis (Figure 1E). Since F-actin highly accumulated at the bottom of cells during these stages (Figures 2C-E), it is thought to be necessary for germlings to grow upright, and for the maintenance of cell axis.

Involvement of Phosphatidylinositol Signaling in the Establishment and Maintenance of Cell Polarity in Monospores

Phosphoinositides (PIs) are derivatives of phosphatidylinositol (PtdIns) and are involved in a wide variety of physiological regulation of the cytoskeleton, vesicle trafficking, ion channels and ion pumps (Zonia and Munnik, 2006). Many works have accumulated evidence regarding the role of PtdIns kinases and phospholipases in the distribution of PI derivatives (e.g., phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2] and phosphatidylinositol-3,4,5-trisphosphate [PtdIns(3,4,5)P_3]) in migrating cells. This section highlights the involvement of PI signaling in the formation of cell polarity in monospores, and provides brief summaries of those in *Dictyostelium* and mammalian cells.

Phosphatidylinositol 3-Kinase (PI3K)

The response of *Dictyostelium* cells to chemoattractants results in he formation of a new leading edge in which preferential activation of PI 3-kinase (PI3K) to produce PtdIns(3,4,5)P_3 on the side facing the chemoattractant gradient is necessary for polarized F-actin localization and directional movement (Janetopoulos and Firtel, 2008). In contrast, phosphatase and Tensin homolog (PTEN) and Src homology 2 domain-containing inositol-5-phosphatase 1 (SHIP1), both of which dephosphorylate PtdIns(3,4,5)P_3, are localized on the trailing edge where they act as negative regulators of PI3K signaling in *Dictyostelium* cells and neutrophils (Funamoto *et al*., 2000; Nishio *et al*., 2007). Localized distributions of PI3K and PtdIns(3,4,5)P_3 phosphatase therefore help cells define their polarity by organizing polarized localization of F-actin. In plants, the importance of PI3K was also demonstrated in polar tip growth of root hairs (Lee *et al*., 2008). However, little is known about the kinds of D-3 PIs involved in tip growth.

It has recently been demonstrated that PI3K activity is required for the establishment of cell polarity, leading to asymmetrical localization of F-actin in migrating monospores (Li *et al*., 2008). Since the use of LY294002, a PI3K inhibitor, prevented monospore migration, it was concluded that the PI3K activity is essential for the establishment of cell polarity and asymmetric distribution of F-actin for migration. These results are similar to those observed in *Dictyostelium* cells and leukocytes.

Of the three types of PI3Ks, the type I PI3K is responsible for the production of PtdIns(3,4,5)P_3 in *Dictyostelium* and mammalian cells (Funamoto *et al*., 2002). Although the PtdIns3P-producing type III PI3K is found in plants (Michell, 2008), plant genomes have no gene encoding the type I PI3K. This is consistent with the fact that PtdIns(3,4,5)P_3 have not yet been detected in any plant cell (Mueller-Roeber and Pical, 2002). Interestingly, yeast cells, which also lack PtdIns(3,4,5)P_3, can produce PtdIns(3,4,5)P_3 in a type III PI3K-dependent manner, when their PTEN-homologue was inactivated by gene disruption (Mitra *et al*., 2004). It is still unclear whether PtdIns(3,4,5)P_3 exists and corresponds to the LY29400-sensitive D3-phosphorylated PtdIns in *P. yezoensis*.

Phospholipase C (PLC) Signaling Cascade

Phospholipase C (PLC) hydrolyzes PtdIns(4,5)P_2 to produce two second messengers, diacylglycerol (DG) and inositol-1,4,5-trisphoshphate (IP_3). These messengers in turn activate protein kinase C and facilitate the release of Ca^{2+} from intracellular stores via the IP_3 receptor (IP3R) (Berridge and Irvine, 1984). PLC is involved in chemotaxis in T cells via an increase in Ca^{2+} from intracellular stores by IP3R (Bach *et al*., 2007). In addition, during cAMP-dependent chemotaxis in *Dictyostelium* cells, PLC is thought to control the concentration of PtdIns(4,5)P_2 that is phosphorylated by PI3K to produce PtdIns(3,4,5)P_3, which is involved in chemotaxis (Kortholt *et al*., 2007). Thus, PLC has two different roles: the regulation of Ca^{2+}-dependent downstream signaling via IP3R, and determination of the PtdIns(4,5)P_2 concentration involved in the activation of PI3K signaling.

Li *et al.* (2009) have demonstrated the involvement of PLC in the establishment of cell polarity in monospores. In the presence of U73122, a specific inhibitor of PLC, monospores did not start moving when the asymmetrical distribution of F-actin (Figure 3B) was inhibited. This was further confirmed by the use of the inactive analog U73343, which did not affect monospore motility. These results indicate that PLC is involved in the establishment of cell polarity to direct the asymmetrical localization of the F-actin of monospores.

In plants, DG produced by PLC is immediately converted by diacylglycerol kinase (DGK) to phosphatidic acid (PA), an important second messenger involved in various physiological processes in plant cells (Zonia and Munnik, 2006). When the role of DGK in the polarity formation of monospores was tested using a DGK inhibitor R59022, migration and asymmetrical distribution of F-actin in monospores were inhibited (Figure 3C). Moreover, the requirement for IP3R-like activity in monospore migration was examined using an IP3R antagonist, 2-APB. This antagonist inhibits IP3R activity on the ER membrane in animal cells. In the presence of this antagonist, both monospore migration and asymmetrical distribution of F-actin were prevented (Figure 3D). Thus, DGK and IP3R-like protein are possibly involved in the establishment of cell polarity in monospores (Li *et al*., 2009), which is consistent with the effects of PLC. DGK involvement was also demonstrated in polarization and polar growth of zygotes in the brown alga *Silvetia compressa* (Peters *et al*., 2008).

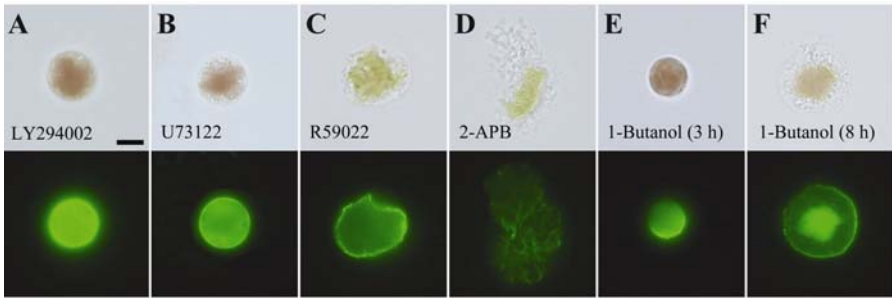

Figure 3. Effects of inhibitors on polarized F-actin accumulation during early development of monospores.
F-actin was stained with Alex Flour 488 phalloidin after incubation with inhibitors for 3 hours and with 1-Butanol for 8 hours. Upper and lower photos in each panel show bright and fluorescent field images, respectively. Monospores treated with chemicals became too weak to bear the weight of the glass cover slip. Changes in the color of chloroplasts and/or enhanced fluorescence signal from chloroplasts sometimes occurred as the monospores were crushed by the cover slips (Li *et al*., 2008; Li *et al*., 2009). (a) Monospore treated with 0.2 mM Cyt B, which cleaves actin filaments. (b) Monospore treated with 25 μM Lat B, which impairs polymerization of G-actin. (c) Monospore treated with 15 μM LY294002, which inhibits PI3K. (d) Monospore treated with 1 μM U73122, which inhibits PLC. (e) Monospore treated with 15 μM R59022, which inhibits DGK. (f) Monospore treated with 20 μM 2-APB, which inhibits IP3R. (g) Monospore treated for 3 hours with 0.4% 1-Butanol, which inhibits PLD (h) Monospore treated with 0.4% 1-Butanol for 8 hours. Scale bar = 5 μm.

In land plants, the presence and nature of IP3R, which acts as an IP_3-dependent Ca^{2+} channel on vacuolar and/or ER membranes, have yet to be determined. Indeed, no IP3R genes bearing a homology to animal genes have so far been found in the genomes of *Arabidopsis thaliana*, rice and *Physcomitrella patens*. In contrast, IP3R homologues have been identified in green algae *Chlamydomonas reinhardtii* and *Volvox carterii*, suggesting the loss of IP3R by land plants when they diverged (Wheeler and Brownlee, 2008). Thus, it is possible that red algae also have orthotic IP3R, since green and red algae originated from the same single ancestor (Palmer, 2000; McFadden and van Dooren, 2004). Identification of IP3R in *P. yezoensis* will be of further importance in understanding the PI signaling system in migrating monospores.

Phospholipase D (PLD)

Phospholipase D (PLD) catalyzes the production of PA from phosphatidylcholine (PC) in a PtdIns(4,5)P_2-dependent manner (Hodgkin *et al*., 2000)**.** PLD inhibition resulted in a rapid decrease in PtdIns(4,5)P_2 synthesis, leading to defects in actin-based motility in *Dictyostelium* cells (Zouwail *et al*., 2005). PLD activity has also been shown to regulate microtubule organization for cell polarity determination in Fucoid zygotes (Peters *et al*., 2007). Moreover, PtdIns(4,5)P_2-dependent PLD activity is involved in tip growth of pollen tubes (Potocký *et al*., 2003). These findings suggest that the PtdIns(4,5)P_2-dependent activation of PLD is important for motility regulation.

Involvement of PLD in the formation of cell polarity has also been demonstrated in monospores (Li *et al*., 2009). Treatment of monospores with 1-butanol for three hours decreased migration, whereas F-actin was asymmetrically localized (Figure 3E). However, treatment of monospores for eight hours with 1-butanol resulted in symmetrically distributed F-actin without migration (Figure 3F). This observation indicates that inhibition of PLD activity did not disrupt the formation of F-actin asymmetry but prevented its maintenance. Thus, PLD participates in the maintenance, but not in the establishment, of cell polarity in monospores.

Conclusion

The establishment and maintenance of cell polarity during migration of monospores is under complex regulation. As discussed above, inhibition of the establishment of cell polarity, as judged by the ability of F-actin to localize asymmetrically, occurred when monospores were treated with inhibitors of PI3K, PLC, DGK and IP3R (Figure 3). In contrast, PLD inhibition prevented monospore migration but not asymmetrical localization of F-actin (Figure 3). Therefore, there is functional diversity between the PLC and PLD signaling systems in terms of the formation of cell polarity; the former being critical for the establishment of cell polarity and the latter playing a role in the maintenance of established cell polarity (Figure 4). Recently, it has been demonstrated that Ca^{2+} influx is indispensable for asymmetric localization of F-actin and migration in monospores (Li *et al*., 2009). These results indicate

the involvement of Ca^{2+}-dependent activation of PI3K and PLC to establish cell polarity (Figure 4). In light of these findings and related literature, it appears that the mechanisms mediating the formation of cell polarity in migrating eukaryotic cells converge into conserved PI signaling pathways. Therefore, dissecting these molecular mechanisms could help in further understanding the interrelationship between PI signaling and F-actin asymmetry, which can provide new insights into the machinery regulating the formation of cell polarity in eukaryotes.

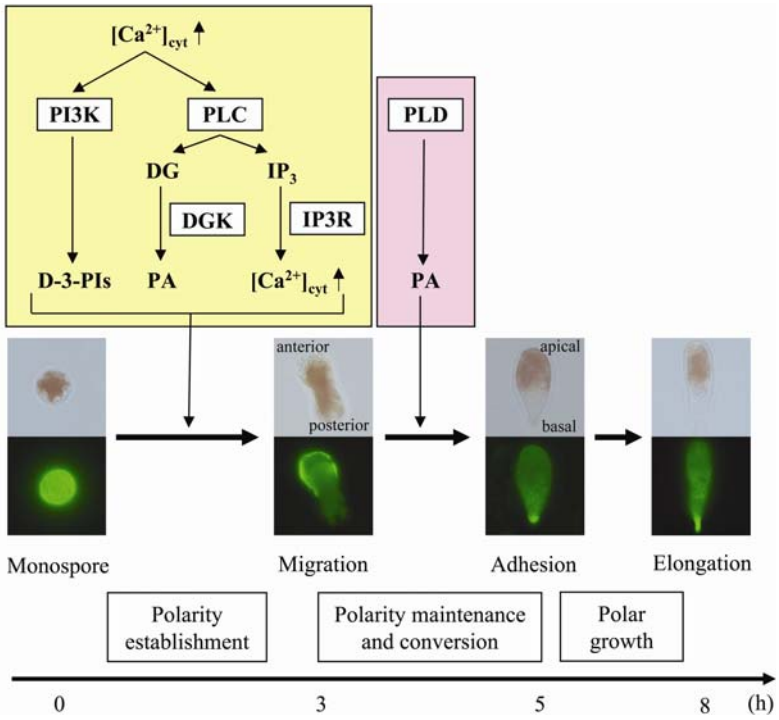

Figure 4. A model of the involvement of PI signaling in the formation of cell polarity in *P. yezoensis* monospores.
Ca^{2+}-dependent activation of PI3K and PLC directs the establishment of the anterior-posterior axis that leads to asymmetrical organization of F-actin, which is localized at the leading edge, thus providing the force for migration. PLD is required for the maintenance of established cell polarity. The anterior-posterior axis is converted to the apical-basal axis as the cell adheres to the substratum, causing localized accumulation of F-actin at the bottom of the monospore; this is important for the maintenance of apical-basal polarity and succeeding polar growth.

Further studies are needed to elucidate the function of individual PIs in the formation of cell polarity in migrating monospores. The ability to visualize the subcellular localization of PI-binding protein domains fused to fluorescent proteins would be a powerful tool for understanding the physiological importance and roles of PIs. Recently, we have developed a system for the efficient expression of humanized fluorescent proteins in *P. yezoensis* cells. This system was successfully employed in visualizing the localization of human Pleckstrin homology domains in plasma membranes and the nuclear localization of transcription factors (Mikami *et al*., 2009; Uji *et al*., 2009). However, overexpression of humanized fluorescent proteins had an inhibitory effect on the development of monospores (Mikami *et al*., 2009; Uji *et al*., 2009), which prevented the analysis of the molecular mechanisms regulating the development of monospores. The visualization of asymmetrical distribution of PIs is critical for understanding how monospore migration is regulated by PI signaling systems. Therefore, it is necessary to remove the inhibitory effects of overexpressed fluorescent proteins on monospore development. Such an approach could reveal how PI-regulated F-actin asymmetry is conserved in migrating eukaryotic cells.

Acknowledgments

I would like to thank my colleagues for valuable discussions, and especially Lin Li and Megumu Takahashi for sharing their unpublished works and assistance in the preparation of figures.

References

Ackland, J. C., West, J. A. & Pickett-Heaps, J. (2007). Actin and myosin regulate pseudopodia of *Porphyra pulchella* (Rhodophyta) archeospores. *Journal of Phycology*, *43*, 129-138.

Bach, T. L., Chen, Q. M., Kerr, W. T., Wang, Y., Lian, L., Choi, J. K., Wu, D., Kazanietz, M. G., Koretzky, G. A., Zigmond, S. & Abrams, C. S. (2007). Phospholipase Cβ is critical for T cell chemotaxis. *The Journal of Immunology*, *179*, 2223-2227.

Berridge, M. J. & Irvine, R. F. (1984). Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*, *312*, 315-321.

Coelho, S. M., Peters, A. F., Charrier, B., Roze, D., Destombe, C., Valero, M. & Cock, J. M. (2007). *Complex life cycles of multicellular eukaryotes*: *new approaches based* on the use of model organisms. *Gene*, *406*, 152-170.

Fu, Y., Wu, G. & Yang, Z. (2001). Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tube. *The Journal of Cell Biology*, *152*, 1019-1032.

Funamoto, S., Meili, R., Lee, S., Parry, L. & Firtel, R. A. (2002). Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell*, *109*, 611-623.

Hepler, P. K., Vidali, L. & Cheung, A. Y. (2001). Polarized cell growth in higher plants. *Annual Review of Cell and Developmental Biology*, *17*, 159-187.

Hodgkin, M. N., Masson, M. R., Powner, D., Saqib, K. M., Ponting, C. P. & Wakelam, M. J. O. (2000). Phospholipase D regulation and localisation is dependent upon a phosphatidylinositol 4,5-bisphosphate-specific PH domain. *Current Biology*, *10*, 43-46

Iglesias, P. A. & Devreotes, P. N. (2008) Navigating through models of chemotaxis. *Current Opinion in Cell Biology*, *20*, 35-40.

Janetopoulos, C. & Firtel, R. A. (2008) Directional sensing during chemotaxis. *FEBS Letters*, *582*, 2075-2085.

Jedd, G. & Chua, N. H. (2002). Visualisation of peroxisomes in living plant cells reveals acto-myosin-dependent cytoplasmic streaming and peroxisome budding. *Plant and Cell Physiology*, *43*, 384-392.

Klinger, T. (1993). The persistence of haplodiploidy in algae. *Trends in Ecology and Evolution*, *8*, 256-258.

Kortholt, A., King, J. S., Keizer-Gunnink, I., Harwood, A. J. & van Haastert, P. J. M. (2007). Phospholipase C regulation of phosphatidylinositol 3,4,5-trisphosphate-mediated chemotaxis. *Molecular Biology of the Cell*, *18*, 4772-4779.

Lee, Y., Bak, G., Choi, Y., Chuang, W.I., Cho, H.T. & Lee, Y. (2008) Roles of phosphatidylinositol 3-kinase in root hair growth. *Plant Physiology*, *147*, 624-635.

Li, L., Saga, N. & Mikami, K. (2008). Phosphatidylinositol 3-kinase activity and asymmetrical accumulation of F-actin are necessary for establishment of cell polarity in the early development of monospores from the marine

red alga *Porphyra yezoensis*. *Journal of Experimental Botany*, *59*, 3575-3586.

Li, L., Saga, N. & Mikami, K. (2009). Ca^{2+} influx and phosphoinositide signaling are essential for the establishment and maintenance of cell polarity in monospores from the red alga *Porphyra yezoensis*. *Journal of Experimental Botany*, *60*, 3477-3489.

McFadden, G. I. & van Dooren, G. G. (2004). Evolution: red algal genome affirms a common origin of all plastids. *Current Biology*, *14*, R514-R516.

Michell, R. H. (2008). Inositol derivatives: evolution and functions. *Nature Reviews Molecular Cell Biology*, *9*, 151-61.

Mikami, K., Uji, T., Li, L., Takahashi, M., Yasui, H. & Saga, N. (2009). Visualization of phosphoinositides via the development of the transient expression system of a cyan fluorescent protein in the red alga *Porphyra yezoensis*. *Marine Biotechnology*, *11*, 563-569.

Mitra, P., Zhang, Y., Rameh, L. E., Ivshina, M. P., McCollum, D., Nunnari, J. J., Hendricks, G. M., Kerr, M. L., Field, S. J., Cantley, L. C. & Ross, A. H. (2004). A novel phosphatidylinositol(3,4,5)P_3 pathway in fission yeast. *Journal of Cell Biology*, *166*, 205-211.

Mueller-Roeber, B. & Pical, C. (2002). Inositol phospholipid metabolism in *Arabidopsis*. Characterized and putative isoform of inositol phospholipid kinase and phosphoinositide-specific phospholipase C. *Plant Physiology*, *130*, 22-46.

Nishio, M., Watanabe, K., Sasaki, J., Taya, C., Takasuga, S., Iizuka, R., Balla, T., Yamazaki, M., Watanabe, H., Itoh, R., Kuroda, S., Horie, Y., Förster, I., Mak, T. W., Yonekawa, H., Penninger, J. M., Kanaho, Y., Suzuki, A. & Sasaki, T. (2007). Control of cell polarity and motility by the PtdIns(3,4,5)P_3 phosphatase SHIP1. *Nature Cell Biology*, *9*, 36-44.

Palmer, J. D. (2000). Molecular evolution: A single birth of all plastids? *Nature*, *405*, 32-33.

Peters, N. T., Logan, K. O., Miller, A. C. & Kropf, D. L. (2007). Phospholipase D signaling regulates microtubule organization in the fucoid alga *Silvetia compressa*. *Plant and Cell Physiology*, *48*, 1764-1774.

Peters, N. T., Pol, S. U. & Kropf, D. L. (2008). Phospholipid signaling during stramenopile development. *Plant Signaling & Behavior*, *3*, 398-400.

Pickett-Heaps, J. D., West, J. A., Wilson, S. M. & McBride, D. L. (2001). Time-lapse videomicroscopy of cell (spore) movement in red algae. *European Journal of Phycology*, *36*, 9-22.

Potocký, M., Eliás, M., Profotová, B., Novotná, Z., Valentová, O. & Zárský, V. (2003). Phosphatidic acid produced by phospholipase D is required for tobacco pollen tube growth. *Planta*, *217*, 122-130.

Saga, N. & Kitade, Y. (2002). *Porphyra*: A model plant in marine science. *Fisheries Science*, *68*, S1075-S1078.

Samaj, J., Peters, M., Volkmann, D. & Baluska, F. (2000). Effects of myosin ATPase inhibitor 2,3-butanedione 2-monoxime on distributions of myosins, F-actin, microtubules, and cortical endoplasmic reticulum in maize root apices. *Plant and Cell Physiology*, *41*, 571-582.

Sieberer, B. J., Ketelaar, T., Esseling, J. J. & Emons, A. M. (2005). Microtubules guide root hair tip growth. *New Phytologist*, *167*, 711-719.

Smith, L.G. (2003). Cytoskeletal control of plant cell shape: Getting the fine points. *Current Opinion in Plant Biology*, *6*, 63-73.

Staiger, C. J. (2000). Signaling to the actin cytoskeleton in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *51*, 257-288.

Uji, T. Takahashi, M, Saga, N. & Mikami, K. (2009). Visualization of nuclear localization of transcription factors with cyan and green fluorescent proteins in the red alga *Porphyra yezoensis*. *Marine Biotechnology*, DOI 10/107/s10126-009-9210-5.

Wheeler, G. L. & Brownlee, C. (2008). Ca^{2+} signalling in plants and green algae-changing channels. *Trends in Plant Science*, *13*, 506-514.

Zonia, L. & Munnik, T. (2006). Cracking the green paradigm: Functional coding of phosphoinositide signals in plant stress responses. *Subcellular Biochemistry*, *39*, 207-237.

Zouwail, S., Pettitt, T. R., Dove, S. K., Chibalina, M. V., Powner, D. J., Haynes, L., Wakelam, M. J. & Insall, R. H. (2005). Phospholipase D activity is essential for actin localization and actin-based motility in *Dictyostelium*. *Biochemical Journal*, *389*, 207-214.