Plant Cell Physiol. 43(5): 479–483 (2002)

Rhizoid Differentiation in *Spirogyra*: Position Sensing by Terminal Cells

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Some species of *Spirogyra* anchor themselves to the substrate by differentiating rhizoids. A rhizoid is differentiated only from the terminal cell, suggesting that this cell can recognize its terminal position in a filament. In the present study, we have analyzed the mechanism for position sensing by the terminal cell. When a filament is cut, a new cell occupies the terminal position, and three phenomena are induced: (1) the cell wall of the cut cell detaches from the new terminal cell; (2) adhesive material is secreted by the terminal cell; and (3) the terminal cell begins to differentiate a rhizoid via tip growth. All of these phenomena were inhibited by adding sorbitol to the external medium, suggesting that turgor pressure is involved in position sensing by the terminal cell. The inhibition by sorbitol was reversible. Upon cutting a filament, the distal end of a new terminal cell became convex. However, when a filament was cut in the presence of sorbitol, the distal end of a new terminal cell became less convex. Either treatment with Gd^{3+} or decrease in extracellular Ca^{2+} resulted in inhibition of all these phenomena, suggesting possible involvement of stretch-activated ion channel in position sensing by terminal cells.

Key words: Calcium — Rhizoid — *Spirogyr*a — Stretch-activated channel.

Abbreviations: APW, artificial pond water; $[Ca^{2+}]$, Ca^{2+} concentration in the external medium; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; FL-BSL, fluorescein-*Bandeiraea* (*Griffonia*) *simplicifolia* lectin; LBM, lectin-binding material; SA channel, stretch-activated channel.

Introduction

Some species of *Spirogyra* differentiate rhizoids from terminal cells of filaments, and these cells attach the filaments to the substratum. Light is the key factor in initiation of rhizoid differentiation, and phytochrome acts as the photoreceptor (Nagata 1973b, Nagata 1979). When a filament is cut, the cell wall of the cut cell detaches from the new terminal cell, and adhesive materials are secreted at the tip of this cell. The growth mode of the cell changes from diffuse growth to tip growth, and finally rhizoids are differentiated without cell division taking place (Nagata 1973a). Nagata (1977) had previously reported that transparent material, supposed to be adhesive, was secreted by the cell from which the rhizoid differentiates. We investigated this phenomenon in our previous study (Inoue et al. 1999), and found that the secreted material was selectively stained by a fluorescently labeled lectin, *Bandeiraea* (*Griffonia*) *simplicifolia* lectin I, indicating that the transparent material contains sugar. The staining reaction was very sensitive and thus we could detect the very early stages of secretion.

Since rhizoids differentiate only from terminal cells (Nagata 1973a, Inoue et al. 1999), we suggested that the terminal cell can recognize its own position in a filament. In the present study, we have focused our attention on the mechanism by which this occurs. The targets of our analysis were the three phenomena induced when a filament is cut: detachment of the cell wall of the cut cell from the new terminal cell; the secretion of adhesive material by this cell; and the change in growth mode from diffuse to tip growth, which signals the commencement of rhizoid differentiation.

Results

After cutting, filaments were incubated in modified Reichart's medium and periodically examined under the microscope. One day after cutting, detachment of the cell wall, secretion of lectin-binding material, and tip growth (an indicator of the commencement of rhizoid differentiation), were already observed in most terminal cells (Fig. 1).

Filaments were incubated in Reichart's medium supplemented with either 0.1, 0.2 or 0.3 M sorbitol for 3 d in order to examine the influence of cell turgor pressure. Cells could only survive in a medium containing 0.4 M sorbitol for short periods, and so, only results obtained in the range of 0–0.3 M sorbitol concentration are included. Detachment of the cell wall of the cut cell from the terminal cell, secretion of the lectin-binding material (LBM) and the start of tip growth were all inhibited by sorbitol in a concentration-dependent manner (Fig. 2).

We then determined whether or not the effect of sorbitol was reversible (Fig. 3). Filaments were incubated in artificial pond water (APW) instead of Reichart's medium because the simple composition of APW facilitated the preparation of incubation media containing various chemicals. The three phenomena accompanying rhizoid differentiation occurred normally in

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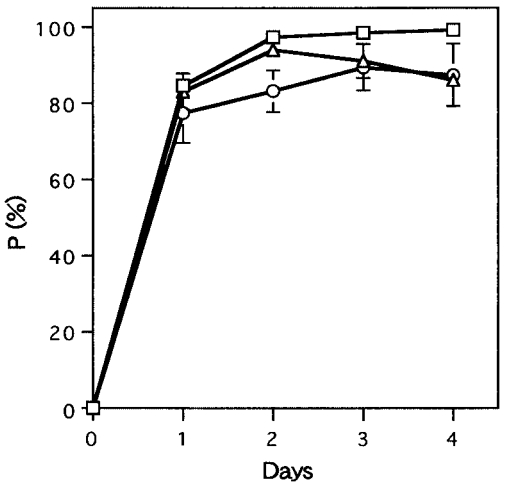

Fig. 1 The time course of rhizoid differentiation. Filaments were incubated in Reichart's medium under 12 h light/12 h dark condition at 23°C. Experiments were repeated five times and average values are shown with SD. Symbols indicate; detachment of the cell wall of the cut cell (open square), secretion of lectin-binding material (open triangle), and start of tip growth (open circle). Abscissa represents time after cutting filaments. P(%) = % of cells showing phenomenon/all cells examined.

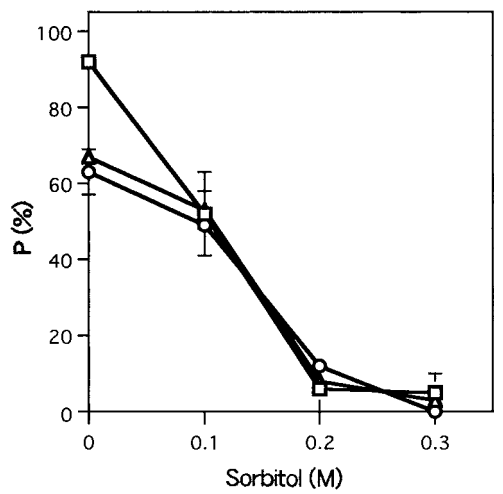

Fig. 2 The effect of sorbitol on rhizoid differentiation. Filaments were incubated in Reichart's medium supplemented with sorbitol of various concentrations for 3 d. Experiments were repeated five times and average values are shown with SD. Symbols, abscissa and P(%) are the same as in Fig. 1.

APW. None of the phenomena associated with rhizoid differentiation were induced in filaments that were incubated for 1 d in APW containing 0.3 M sorbitol (Fig. 3). Filaments that had been incubated for 1 d in 0.3 M sorbitol/APW were then transferred to APW lacking sorbitol. The sorbitol concentration was decreased gradually (at a rate of 0.1 M min^{-1}) to avoid the cell damage that a sudden increase in turgor pressure might cause. All three phenomena were induced in the sorbiol-free APW

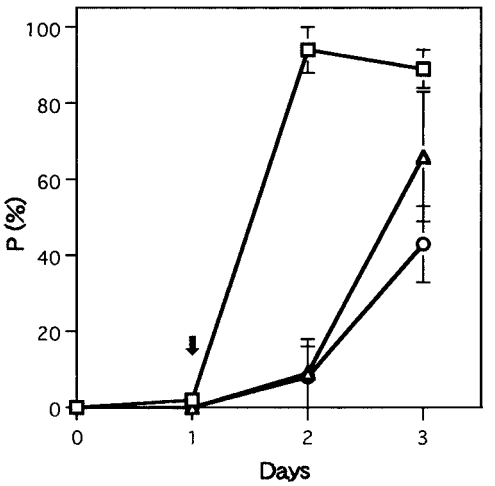

Fig. 3 The reversibility of the sorbitol effect. Filaments were incubated in APW supplemented with 0.3 M sorbitol for 1 d, and then the external medium was changed to APW lacking sorbitol (arrow). Experiments were repeated five times and average values are shown with S.D. Symbols, abscissa and P (%) are the same as in Fig. 1.

(Fig. 3). Detachment of the cell wall of the cut cell was the first phenomenon to occur.

We observed a significant morphological change after cutting filaments. Prior to cutting, the septum separating adjacent cells was flat, probably because the turgor pressure of the cells on either side was balanced (Fig. 4a arrowhead). When a filament was cut in APW, the distal septum of the new terminal cell was exposed to the external medium and then became convex (Fig. 4b; Nagata 1973a). When a filament was cut in 0.3 M sorbitol/APW, the extent of convexity of the distal septum was similar to that of a filament cut in APW alone (Fig. 4c). However, the distal septum was less convex when a filament was cut in 0.4 M sorbitol/APW (Fig. 4d), probably because the turgor pressure of the cell had decreased.

The effect of Gd^{3+}, an inhibitor of stretch-activated channel (Garrill et al. 1993), was studied. Filaments were cut into fragments of 1–3 mm in length, transferred into APW supplemented with $GdCl_3$ of various concentrations and incubated for 1 d. Gd^{3+} sensitively inhibited three phenomena accompanying rhizoid formation. Detachment of cell wall was less sensitive but it was completely inhibited with 20 μM $GdCl_3$ (Fig. 5). Reversibility of the effect of Gd^{3+} was studied. However, results were very variable among experiments. In some experiments, three processes were recovered in most filaments, but in other experiments, these processes were not recovered at all (data not shown). The reason for this variation of the results is remained unsolved.

Next, we investigated the effect of the external Ca^{2+} concentration ($[Ca^{2+}]$). When $[Ca^{2+}]$ was drastically decreased, cells were damaged and died during incubation. However, addition of 2 mM $MgCl_2$ relived cells from severe damage by

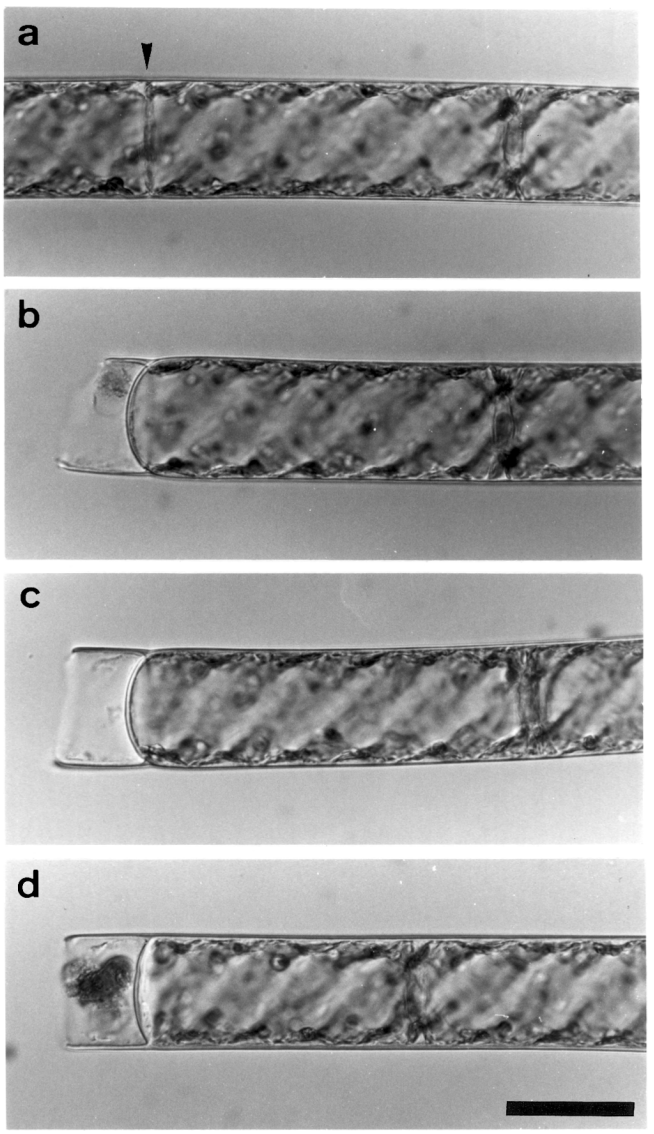

Fig. 4 Morphology of the septa of filaments. (a) Two cells of a filament in APW, prior to cutting. The cross wall was planar (arrowhead). (b) Following cutting, in APW alone, the distal septum of a terminal cell became convex. (c) The distal septum of a terminal cell also became convex when the filament was cut in the presence of 0.3 M sorbitol/APW. (d) The convexity of the distal septum of a terminal cell was reduced, following cutting of a filament in 0.4 M sorbitol/APW. Bar, 50 μm.

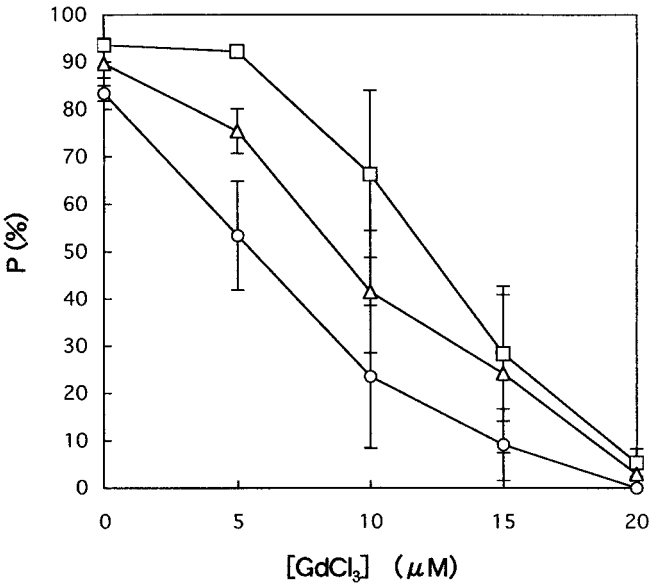

Fig. 5 Effect of Gd^{3+} on rhizoid differentiation. Filaments were cut into fragments of 1–3 mm in length, transferred into APW supplemented with $GdCl_3$ of various concentrations and incubated for 1 d. Experiments were repeated seven times and average values are shown with SD. Symbols and P(%) are the same as in Fig. 1.

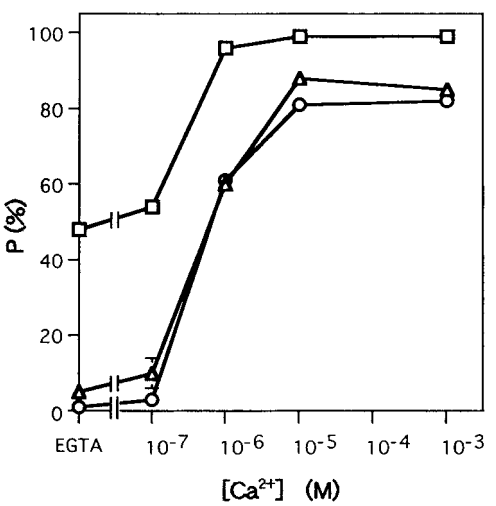

Fig. 6 Effect of Ca^{2+} on rhizoid differentiation. Filaments were incubated in APW supplemented with 1 mM EGTA, 1 mM HEPES, 2 mM $MgCl_2$ and $CaCl_2$ of various concentrations for 1 d. Experiments were repeated six times and average values are shown with SD. Symbols and P(%) are the same as in Fig. 1.

lowering $[Ca^{2+}]$. Therefore, all experiments were carried out in the presence of 2 mM $MgCl_2$. At 10^{-5} M $[Ca^{2+}]$, three phenomena were induced normally. However, further decrease in $[Ca^{2+}]$ induced significant inhibition of the LBM secretion and start of tip growth (Fig. 6). Detachment of cell wall was induced even at very low $[Ca^{2+}]$, suggesting that Ca^{2+} is involved in binding between cell walls of neighboring cells.

Reversibility of inhibition by lowering $[Ca^{2+}]$ was examined (Fig. 7). When filaments were incubated in a medium containing 2 mM $MgCl_2$ and 1 mM EGTA for 1 d, secretion of LBM and start of tip growth were severely inhibited. Detachment of the cut cell wall was observed in about 70% cells (a). After treatment in the above EGTA medium for 1 d, filaments were transferred into APW and incubated for further 1 d. All three phenomena were induced to the extent (b) similar to that of filaments incubated in APW for 2 d (c).

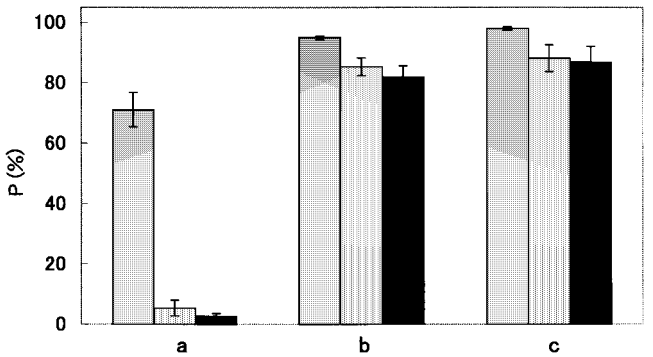

Fig. 7 Reversibility of EGTA effect. Filaments were incubated in Ca^{2+}-free EGTA solution for 1 d (a). Filaments were incubated in Ca^{2+}-free EGTA solution for 1 d then in APW for 1 d (b). Filaments were incubated in APW for 2 d (c). Experiments were repeated five times and average values are shown with SD. Columns indicate detachment of the cell wall of the cut cell (left), secretion of lectin-binding material (center), and start of tip growth (right).

Discussion

Our results strongly suggest that the turgor pressure of a cell is intimately involved in the induction of the phenomena associated with rhizoid differentiation. When the cell turgor pressure was decreased by adding sorbitol to the external medium, all three phenomena accompanying rhizoid differentiation were inhibited (Fig. 2). Three phenomena were completely inhibited by 0.3 M sorbitol (Fig. 2), although the extent of convexity of the distal septum in 0.3 M sorbitol was similar to that in APW lacking sorbitol (Fig. 4). This can be explained in terms of the mechanical properties of the cell wall at the distal end. That is, the cell wall may be stretched to its fullest extent by a small osmotic stress in 0.3 M sorbitol. Although the stretching of the membrane at the distal end is almost isometric between 0 and 0.3 M sorbitol, the mechanical stress experienced by the membrane in APW lacking sorbitol should be significantly greater than that in 0.3 M sorbitol/APW. The inhibition of the three phenomena by sorbitol was reversible (Fig. 3). When sorbitol was removed, the cell walls of the cut cells began to detach rapidly, but the other two processes were rather slow. Whilst the initial signal that induces the three phenomena may be the same, the transduction of this signal may occur via different pathways for each process.

The development of a convex distal septum in the terminal cell suggests that the plasma membrane in this region was stretched (Fig. 4). Thus, stretching of the plasma membrane (or high stress) at the distal end may well be the signal that induces rhizoid differentiation. The fact that the rhizoid is formed at the distal end also supports the idea that sensing of a cell's terminal position is carried out at the distal septum. A number of authors have reported the presence of ion channels activated by stretching of the plasma membrane in plant cells (Cosgrove and Hedrich 1991, Falke et al. 1988, Garrill et al. 1992, Garrill et al. 1993, Ding and Pickard 1993a, Ding and Pickard 1993b, Ding et al. 1993). Inhibition by Gd^{3+} suggested involvement of stretch-activated channels in perceiving the deformation of the plasma membrane (or high stress) at the distal end of the cell (Fig. 4).

Since decrease in the extracellular Ca^{2+} significantly inhibited the targeting phenomena (Fig. 6), the role of Ca^{2+} influx was suggested. It has been reported that intracellular Ca^{2+} is indispensable for tip growth. A tip-focused intracellular Ca^{2+} gradient and a tip-localized influx of the extracellular Ca^{2+} are required for pollen tube growth (Li et al. 1999, Holdaway-Clarke et al. 1997, Nobiling and Reiss 1987, Obermeyer and Weisenseel 1991, Rathore et al. 1991, Miller et al. 1992, Pierson et al. 1994, Pierson et al. 1996) and root hair growth in *Arabidopsis* (Bibikova et al. 1997). Using the patch clamp technique, the presence of a stretch-activated (SA) channel permeable to Ca^{2+} in the plasma membrane at the tip of hyphae of *Saprolegnia ferax* has been reported (Garrill et al. 1992, Garrill et al. 1993, Levina et al. 1994). Gd^{3+} inhibited SA channel activity and stopped tip growth (Garrill et al. 1993). It seems that the SA channel permeable to Ca^{2+} plays an important role in tip growth. The situation might be also the case in differentiation of rhizoid in *Spirogyra*.

Wounding induces various morphological change in surrounding cells, induction of tracheary element (cited in Fukuda 1992), migration of nucleus (Schnepf and Volkmann 1974), change in organization of microtubules (Hush et al. 1990, Hush and Overall 1992), and change of division plane (Goodbody and Lloyd 1990). Thus, change of intracellular morphology and induction of differentiation in neighboring cells upon wounding seems to be a common phenomenon in plants. Since higher plants form complex tissues, analysis of signal transduction and cell differentiation are rather difficult. On the other hand, *Spirogyra*, having simple organization, can be a suitable system to analyze cell differentiation upon wounding.

Materials and Methods

Spirogyra sp. was collected from a stream near our laboratory, and cultured axenically in a slightly modified Reichart's medium as described previously (Inoue et al. 1999). Cells were used for experiments within 5 d after inoculating from a stock culture into a fresh culture medium.

Filaments were transferred into either slightly modified Reichart's medium (Nagata 1973a) or APW containing 0.1 mM KCl, 0.1 mM $CaCl_2$, 1 mM NaCl, and 1 mM HEPES (pH 7.0). They were then cut into fragments of 1–3 mm using scissors. The cut filaments were transferred into a chamber made from slide glass and silicon rubber as described previously (Inoue et al. 1999). From now on, a cut filament will be referred to a filament. Filaments were incubated at 23°C under fluorescent lamps (12 h light-12 h dark cycle). The light intensity at the surface of the chamber was 90 μmol m^{-2} s^{-1}. We then analyzed the three phenomena accompanying rhizoid differentiation; (1) detachment of the cell wall of the cut cell, (2) secretion of adhesive material, and (3) commencement of tip growth. Secretion of adhesive material was analyzed by staining filaments with fluorescently labeled lectin (Inoue et al. 1999). Briefly, filaments were incubated for 15 min

in APW containing fluorescein-labelled *Bandeiraea* (*Griffonia*) *simplicifolia* lectin I (Vector Laboratories, Inc., Burlingame, CA, U.S.A.) at a concentration of 4 μg ml^{-1}. After washing with APW, specimens were observed with a fluorescence microscope (BH2-RFCA, Olympus, Tokyo, Japan).

Ca^{2+}-buffered medium was prepared using APW supplemented with 1 mM EGTA and 1 mM HEPES, (pH 7.0), 2 mM $MgCl_2$ and $CaCl_2$ of various concentrations. The concentration of free-Ca^{2+} was calculated using an apparent association constant between Ca^{2+} and EGTA, 4.83×10^{-6} M^{-1} (Jewell and Rüegg 1966).

Three phenomena were examined for more than fifty terminal cells. The results are presented as the percentage (P) of cells displaying the above phenomena out of the total number of terminal cells. All experiments were repeated at least five times and average values were shown with SD.

Acknowledgments

We want to express our cordial thanks to Dr. Virginia Anne Shepherd for her critical reading of this manuscript and discussion.

References

Bibikova, N.T., Zhigilei, A. and Gilroy, S. (1997) Root hair growth in *Arabidopsis thaliana* is directed by calcium and an endogenous polarity. *Planta* 203: 495–505.

Cosgrove, J.D. and Hedrich, R. (1991) Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of *Vicia faba* L. *Planta* 186: 143–153.

Ding, P.J., Badot, M.P. and Pickard, G.B. (1993) Aluminium and hydrogen ions inhibit a mechanosensory calcium-selective cation channel. *Aust. J. Plant Physiol.* 20: 771–778.

Ding, P.J. and Pickard, G.B. (1993a) Mechanosensory calcium-selective cation channels in epidermal cells. *Plant J.* 3: 83–110.

Ding, P.J. and Pickard, G.B. (1993b) Modulation of mechanosensitive calcium-selective cation channels by temperature. *Plant J.* 3: 713–720.

Falke, C.L., Edwards, L.K., Pickard, G.B. and Misler, S. (1988) A stretch-activated anion channel in tobacco protoplasts. *FEBS Lett.* 237: 141–144.

Fukuda, H. (1992) Tracheary element formation as a model system of cell differentiation. *Int. Rev. Cytol.* 136: 289–332.

Garrill, A., Jackson, L.S., Lew, R.R. and Heath, B.I. (1993) Ion channel activity and tip growth: tip-localized stretch-activated channels generate an essential Ca^{2+} gradient in the oomycete *Saprolegnia ferax*. *Euro. J. Cell Biol.* 60: 358–365.

Garrill, A., Lew, R.R. and Heath, B.I. (1992) Stretch-activated Ca^{2+} and Ca^{2+}-activated K^+ channels in the hyphal tip plasma membrane of the oomycete *Saprorelnia ferax*. *J. Cell Sci.* 101: 721–730.

Goodbody, C.K. and Lloyd, W.C. (1990) Actin filaments line up across *Tradescantia* epidermal cells, anticipating wound-induced division planes. *Protoplasma* 157: 92–101.

Holdaway-Clarke, L.T., Feijo, A.J., Hackett, R.G., Kunkel, G.J. and Hepler, K.P. (1997) Pollen tube growth and the intracellular cytosolic calcium gradient oscillate in phase while extracellular calcium influx is delayed. *Plant Cell* 9: 1999–2010.

Hush, M.J., Hawes, R.C. and Overall, L.R. (1990) Interphase microtubule re-orientation predicts a new cell polarity in wounded pea roots. *J. Cell Sci.* 96: 47–61.

Hush, M.J. and Overall, L.R. (1992) Re-orientation of cortical F-actin is not necessary for wound-induced microtubule re-orientation and cell polarity establishment. *Protoplasma* 169: 97–106.

Inoue, N., Sonobe, S., Nagata, Y. and Shimmen, T. (1999) Secretion of lectin-binding material in rhizoid differentiation of *Spirogyra*. *Plant Cell Physiol.* 40: 973–977.

Jewell, B.R. and Rüegg, J.C. (1966) Oscillatory contraction of insect fibrillar muscle after glycerol extraction. *Proc. Roy. Soc. B.* 164: 428–459.

Levina, N.N., Lew, R.R. and Heath, B.I. (1994) Cytoskeletal regulation of ion channel distribution in the tip-growing organism *Saprolegnia ferax*. *J. Cell Sci.* 107: 127–134.

Li, H., Lin, Y., Heath, M.R., Zhu, X.M. and Yang, Z. (1999) Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-localized calcium influx. *Plant Cell* 11: 1731–1742.

Miller, D.D., Callaham, D.A., Gross, D.J. and Hepler, P.K. (1992) Free Ca^{2+} gradient in growing pollen tubes of *Lilium*. *J. Cell Sci.* 101: 7–12.

Nagata, Y. (1973a) Rhizoid differentiation in *Spirogyra* I. Basic features of rhizoid formation. *Plant Cell Physiol.* 14: 531–541.

Nagata, Y. (1973b) Rhizoid differentiation in *Spirogyra* II. Photoreversibility of rhizoid induction by red and far-red light. *Plant Cell Physiol.* 14: 543–554.

Nagata, Y. (1977) Light-induced adhesion of *Spirogyra* cells to glass. *Plant Physiol.* 59: 680–683.

Nagata, Y. (1979) Rhizoid differentiation in *Spirogyra* III. Intracellular localization of phytochrome. *Plant Physiol.* 64: 9–12.

Nobiling, R. and Reiss, H.-D. (1987) Quantitative analysis of calcium gradients and activity in growing pollen tubes of *Lilium longiflorum*. *Protoplasma* 139: 20–24.

Obermeyer, G. and Weisenseel, M.H. (1991) Calcium channel blocker and calmodulin antagonists affect the gradient of free calcium ions in lily pollen tubes. *Eur. J. Cell Biol.* 56: 319–327.

Pierson, E.S., Miller, D.D., Callaham, D.A., Shipley, A.M., Rivers, B.A., Cresti, M. and Hepler, P.K. (1994) Pollen tube growth is coupled to the extracellular calcium ion flux and the intracellular calcium gradient: effect of BAPTA-type buffers and hypertonic media. *Plant Cell* 6: 1815–1828.

Pierson, E.S., Miller, D.D., Callaham, D.A., van Aken, J., Hackett, G. and Hepler, P.K. (1996) Tip-localized calcium entry fluctuates during pollen tube growth. *Dev. Biol.* 174: 160–173.

Rathore, K.S., Cork, R.J. and Robinson, K.R. (1991) A cytoplasmic gradient of Ca^{2+} is correlated with the growth of lily pollen tubes. *Dev. Biol.* 148: 612–619.

Schnepf, E. and Volkmann, K. (1974) Inhibition of traumatotactic movement of nuclei in *Tradescantia* leaf epidermis. II. Effects of heavy water, ethionine, Ca^{++}, and cyclic AMP. *Protoplasma* 81: 313–321.

(Received December 20, 2001; Accepted February 14, 2002)