Short Communication

Synthesis of a Callosic Substance during Rhizoid Differentiation in Spirogyra

Shin-ya Yamada, Seiji Sonobe and Teruo Shimmen

Department of Life Science, Graduate School of Science, Himeji Institute of Technology, Harima Science Park City, Hyogo, 678-1297 Japan

Spirogyra living in running water is anchored to the substratum by rhizoids that form at the ends of the filaments. A new terminal cell differentiates into a rhizoid cell if the filament is injured. The mode of growth changes from diffuse to tip growth when rhizoid differentiation begins. In this study, we found that a callosic substance was synthesized during rhizoid differentiation. Decreasing the cell turgor, lowering extracellular Ca$^{2+}$ or adding Gd$^{3+}$, all inhibited the commencement of rhizoid differentiation as well as synthesis of the callose-like substance at the tip of the terminal cell. A callosic substance was also synthesized during formation of the conjugation tube.

Keywords: Ca$^{2+}$ — Callosic substance — Differentiation — Rhizoid — Spirogyra — Tip growth.

Abbreviation: APW, artificial pond water.

Growth and morphogenesis of plants proceeds via control of both cell division and cell expansion. Higher plants composed of complex tissues are not always suitable material for studying these processes at the cellular level. Aquatic algae, with their simpler morphology, can be more appropriate for such research. Perhaps the best-known example is the use of Fucus and Pelvetia zygotes to study the establishment of cellular polarity (Goodner and Quatrano 1993, Kropf 1997).

A Spirogyra filament is a colony composed of tandem cylindrical cells. Morphological differentiation is not observed amongst cells in a filament. However, some Spirogyra living in running water differentiate rhizoids from the terminal cells and use these to anchor to the substratum. If a filament is injured, the new terminal cells differentiate to form a new rhizoid. Nagata (Nagata 1973a, Nagata 1973b, Nagata 1977, Nagata 1979) studied this process extensively and found that phytochrome is involved in regulating rhizoid differentiation. We recently revived this classic research because extensive progress in the life sciences has made it possible to adopt new approaches (Inoue et al. 1999, Inoue et al. 2002, Yoshida et al. 2003).

One of the early signs of the rhizoid differentiation is a change in the mode of growth from diffuse to tip growth. First, a bulge is formed at the very tip of the terminal cell, and this then extends via tip growth. An elaborate rosette-shaped rhizoid is finally formed (Nagata 1973a, Inoue et al. 1999). In the present study, we found that a callosic substance is formed during rhizoid differentiation in Spirogyra.

Spirogyra collected from a stream near our laboratory was cultured, as reported previously (Inoue et al. 1999). Filaments of Spirogyra were cut into 1–3 mm fragments with scissors and incubated in artificial pond water (APW) containing 0.1 mM KCl, 1 mM NaCl, 0.1 mM CaCl$_2$ and 1 mM HEPES-Na (pH 7.0) at 23°C under a 12 h light (90 μmol m$^{-2}$ s$^{-1}$) – 12 h dark cycle. Fragments thus prepared for induction of rhizoid differentiation will be referred to as filaments from now on. To stain the callosic substance, filaments of Spirogyra were incubated in a medium containing 0.01% (w/v) aniline blue and 50 mM K$_2$HPO$_4$ (pH 8.6) for several min, and observed with an epifluorescence microscope (Olympus, BX50) using excitation light of wavelength between 330 and 380 nm. A barrier filter was not used so that the blue fluorescence of aniline blue and the red fluorescence of chlorophyll could both be observed. Aniline blue is generally used for staining of callose/callosic substances (Currier 1957, Galway and McCully 1987, Radford et al. 1998, Worrall et al. 1992, Tucker et al. 2001). The extent of staining with aniline blue is well correlated with that of expression of callose synthase in plant cells, suggesting that this dye stains 1,3-β-glucan (Hong et al. 2001).

Aniline blue staining was not observed at the region of the new terminal cells immediately after cutting the filaments (Fig. 1 (1a, 1b)). Twelve h after cutting the filaments, the cell wall at the tip region was weakly stained, and a small spot was occasionally observed at the very tip of the terminal cells, although bulging had not yet commenced (Fig. 1 (2)). A bulge formed at the distal end of the terminal cell about 20 h after cutting the filaments, and its surface was stained with aniline blue (Fig. 1 (3)). The entire surface of the cylinder-shaped rhizoid formed 1 d after cutting the filament was strongly stained (Fig. 1 (4)). Only the region of the cell formed after the start of rhizoid differentiation was stained—the distal region did not stain. The rosette-shaped rhizoid appeared following further incubation of the filaments. This indicates that the final stage of differentiation has been reached (Nagata 1973a). Only the region of the rosette was strongly stained in rhizoids formed 2 d after cutting the filaments. The basal region did not stain (Fig. 1 (5)).
Fig. 1  (1) Light micrograph of a new terminal cell just after cutting a filament (1a) and its fluorescence micrograph (1b). Wavelength of excitation light was 330–380 nm. The distal end of the cell was not stained with aniline blue. Red fluorescence originated from chlorophyll. Bar = 50 μm. (2) Fluorescence micrograph of a terminal cell 12 h after cutting a filament. The cell wall at the tip region was weakly stained with aniline blue, and a small spot was occasionally observed at the very tip of the terminal cell. Bar = 50 μm. (3) Fluorescence micrograph of a terminal cell about 20 h after cutting a filament. A bulge formed at the distal end was stained with aniline blue. Bar = 50 μm. (4) Fluorescence micrograph of the cylinder-shaped rhizoid formed 1 d after cutting a filament. The entire surface of the rhizoid was stained with aniline blue. Bar = 50 μm. (5) Fluorescence micrograph of the rosette-shaped rhizoid formed 2 d after cutting a filament. The entire surface of the rhizoid was stained with aniline blue. Bar = 50 μm. (6) Fluorescence micrograph of the terminal cell of a filament incubated in APW supplemented with 1 mM EGTA and 2 mM MgCl₂ for 1 d. Bar = 50 μm. (7) Fluorescence micrograph of the terminal cell of a filament incubated in APW supplemented with 15 μM GdCl₃ for 1 d. Bar = 50 μm. (8) Fluorescence micrograph of a cell starting formation of a conjugation tube. A bulge was significantly stained with aniline blue. (9) Fluorescence micrograph of completed conjugation tubes. Bar = 50 μm.
The following two observations indicated that synthesis of a callosic substance was closely associated with rhizoid differentiation. (1) Staining with aniline blue was observed only in terminal cells and not in other cells of the filaments. (2) The occasional terminal cell failed to differentiate. In such cells, strong staining with aniline blue was not observed at all, and very weak staining occurred only rarely.

The possibility that blue auto-fluorescence was associated with rhizoid differentiation was eliminated by observation of unstained rhizoids. Blue autofluorescence was not observed in unstained cells (data not shown).

In general, a rhizoid forms only from the terminal cell, suggesting that the terminal cell recognizes its own position in the filament. Upon cutting a filament, the distal end of a new terminal cell becomes convex. We concluded that membrane stretching at the distal end signals its position to the new terminal cell, because lowering turgor pressure, and preventing stretching, also inhibits rhizoid differentiation (Inoue et al. 2002). Therefore, we examined the effect of decreased cell turgor on synthesis of the callosic substance. After cutting, the filaments were incubated in APW supplemented with either 100 or 200 mM sorbitol. In the presence of 100 mM sorbitol, synthesis of the callosic substance was observed in almost 50% of the terminal cells. However, staining was observed only in those cells that had commenced tip growth. In the presence of 200 mM sorbitol, neither the commencement of tip growth nor staining with aniline blue was observed in any specimen (data not shown).

The presence of extracellular Ca²⁺ is indispensable for induction of rhizoid differentiation (Inoue et al. 2002). Cells died when EGTA was added in order to lower the external concentration of Ca²⁺. However, cells could survive in the presence of EGTA when the medium was also supplemented with MgCl₂ (Inoue et al. 2002). Neither rhizoid differentiation nor staining of the tip surface occurred in APW supplemented with 1 mM EGTA and 2 mM MgCl₂. However, patches of stain were observed in various places other than the tip region (Fig. 1 (6)). This abnormal synthesis of the callosic substance in the absence of Ca²⁺ is unrelated to rhizoid differentiation.

We suggested that stretch-activated ion channels could be involved in position sensing, based on the fact that Gd³⁺ inhibited the rhizoid differentiation (Inoue et al. 2002). After cutting, filaments were incubated in APW supplemented with either 10 or 15 µM GdCl₃ for 1 d. In the presence of 10 µM GdCl₃, neither rhizoid differentiation nor staining with aniline blue was observed (data not shown). In the presence of 15 µM GdCl₃, very strong staining was occasionally observed at the tip region, although tip growth did not start (Fig. 1 (7)). This could be due to a toxic effect of Gd³⁺, akin to wounding, since wounding induces synthesis of callosic substances (Currier 1957).

Conjugation was occasionally observed during culture of Spirogyra, although we could not control the start of this phenomenon. At the beginning of conjugation, a bulge was formed at the flank of the cell. This was the nascent conjugation tube. The bulge stained with aniline blue (Fig. 1 (8)). Conjugation tubes still stained after conjugation was completed (Fig. 1 (9)).

In higher plants, root hairs and pollen tubes elongate via tip growth. The presence of callose at the tip region of root hairs is controversial as discussed by Currier (1957). However, staining of the tip region with aniline blue has been reported at least in some plants such as Hordeum vulgare, Trianeae bognensis (Currier 1957) and Zea mays (Clarke and McCully 1985). It is also ambiguous whether or not callose is present at the tip region of pollen tubes. Currier (1957) reported that the fluorescence from aniline blue was strongest in the wall near the grain but the tip was relatively invisible in eight species of higher plants. When the pollen tubes of Camellia japonica were labeled with a polyclonal antibody against callose, the tip region was stained significantly. However, when aniline blue was used as the callose stain, the tip region failed to stain, whilst the basal region stained significantly (Hasegawa et al. 1996). The situation is clearer in the case of Spirogyra, where formation of both rhizoids and conjugation tubes starts with a bulging of the cells, and the tip regions of both these structures stain significantly with aniline blue. Clearly, the callosic substance will have some role to play in the formation of these structures.

In higher plants, most vegetative cells elongate via diffuse growth. However, formation of root hairs starts via commencement of tip growth in some epidermal cells. Similarly, rhizoid differentiation starts via modification of the mode of growth from diffuse to tip growth. Thus, differentiation of rhizoids in Spirogyra is a useful model system for studying the control mechanism of the mode of elongation growth. In addition, Spirogyra is a suitable material for studying the mechanism of secretion (Inoue et al. 1999) and the mechanism by which differentiation is controlled, by cell position (Inoue et al. 2002) and by cytoskeletal elements (Yoshida et al. 2003).

References


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