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SHORT COMMUNICATION

Inducible growth mode switches influence *Valonia* rhizoid differentiation

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Abstract Cell differentiation and cell type commitment are an integral part of plant growth and development. Investigations on how environmental conditions affect the formation of shoots, roots, and rhizoids can help illustrate how plants determine cell fate and overall morphology. In this study, we evaluated the role of substratum and light on rhizoid differentiation in the coenocytic green alga, *Valonia aegagropila*. Elongating rhizoids displayed varying growth modes and cell shape upon exposure to different substrata and light conditions. It was found that soft substrata and dark incubation promoted rhizoid elongation via tip growth while subsequent exposure to light prevented tip growth and instead induced swelling in the apical region of rhizoids. Swelling was accompanied by the accumulation of protoplasm in the rhizoid tip through expansion of the cell wall and uninhibited cytoplasmic streaming. Subsequent diffuse growth led to the transformation from slender, rod-shaped rhizoids into spherical thallus-like structures that required photosynthesis. Further manipulation of light regimes caused vacillating cell growth redirections. An elongating *V. aegagropila* rhizoid cell thus appears capable of growth mode switching that is regulated by immediate environmental conditions thereby influencing ultimate cell shape and function. This is the first description of inducible, multiple growth mode shifts in a single intact plant cell that directly impact its differentiation.

Keywords Cytoplasmic streaming · Growth mode · Light · Rhizoid · Thallus · *Valonia aegagropila*

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Introduction

There are approximately 40 known plant cell types with distinct shapes or morphologies that are mostly associated with specialized functions (Martin et al. 2001). In order to attain such states, these cells have to develop into these forms through morphogenesis. Morphogenesis has been generally thought to operate through the deformation of the existing wall and the deposition of a new wall (Harold 2002). This is achieved through the interplay of various elements including turgor pressure and the involvement of the cytoskeleton (Smith and Oppenheimer 2005; Mathur 2006). Polar growth or directional expansion in plant cells occur via diffuse growth (cell extension along the entire cell surface) and tip growth (cell extension restricted in dome-shaped apices such as those of root hairs) (Cosgrove 2000; Martin et al. 2001). In giant-celled algae, a less common mode called band growth has been known to occur in addition to tip growth and diffuse growth (Mine et al. 2008).

The genus *Valonia* is one of the giant-celled marine green algae with large multinucleate cells comprising the primary cells and numerous lateral branch cells produced through lenticular cell formation (Olsen and West 1988). During germination, a primary holdfast functions as the initial anchorage of cells on substrata, followed by several rhizoids formed around the basal area (Chihara 1959; Bold and Wynne 1985). In addition, rhizoid-like tenacular cells form on the surfaces of neighboring lateral branch cells to connect

them and provide close thallus packing and reinforcement (Fritsch 1935; Kanda 1940). Rhizoids can be induced on any area of cells through continuous exposure to hydrophobic surfaces of substrata (Elvira et al. 2012), whereas basal rhizoids readily form around pre-existing ones. Formation of a rhizoid starts with localized accumulation of protoplasmic mass in the exposed area, followed by swelling of the cell wall, septum formation, and elongation of the tip of a conical structure. A rod-shaped cell continues to extend until making contact with a substratum and attaches, at which point its fate can be considered as established. However, before this process is completed, a rhizoid cell can be exposed to a variety of other environmental stimuli that might affect its eventual morphology and cell type commitment. For example, thallus cells have been observed to switch into rhizoids and then to thallus cells in laser-ablated *Fucus* embryos (Berger et al. 1994; Bouget et al. 1998) in a manner that resembles a case of transdetermination. Transdetermination occurs when a cell, in the course of differentiation into an expected cell type, develops into an entirely different type (Maclean and Hall 1987). When a cell undergoes an irreversible switch from a differentiated cell type to another, it is referred to as transdifferentiation, the mechanisms of which have been largely unclear (Sugimoto et al. 2011). During these two developmental processes, the modification in cell architecture and function entails a shift in the current growth mode into a new one; thus, a rhizoid exhibiting tip growth needs to assume diffuse growth when transforming into a round thallus cell. In order to start to understand such a phenomenon, a specimen with suitable characteristics for tracking the subtle changes in growth patterns of cells should be utilized. *Valonia aegagropila*, due to its large cell size and conspicuous rhizoid formation stages, presents as an ideal candidate.

In this study, we used various substrata and light regimes to induce morphological changes in *V. aegagropila* rhizoid cells. We thus report how environmental conditions can be manipulated to repeatedly alter rhizoid cell shape and function through the promotion of alternating tip and diffuse growths. Our results present interesting areas for consideration in elucidating the mechanisms involved in algal cell growth and shape regulation.

Materials and methods

Plant material

Cells used in this study were derived from specimens in the algal culture collection of the Cell Biology Laboratory of Kochi University. Zoospores were isolated from *V. aegagropila* (strain no. 7) thalli collected on February 21, 2006 from Sta. Ana, Cagayan, Philippines, following methods described by Kawai et al. (2005). Germlings generated from mature thalli were grown in Petri dishes containing ca. 150 ml of 1/4-stength Provasoli's enhanced seawater medium (PES) (Provasoli 1968) at 22°C in long-day conditions (14:10 h light/dark (LD)) under white fluorescent lamps (40 μmol photons m^{-2} s^{-1}). Cells approximately 3 weeks old had spherical shape and attached to the inner surfaces of Petri dishes with numerous basal rhizoids. Young thallus cells 3–4 mm in diameter were transferred from the Petri dishes into new incubation vessels (see below) and used for rhizoid transformation experiments. These cells had the capability to produce new rhizoids.

Effect of substratum type on rhizoid elongation

To investigate the effect of type of substratum on rhizoid elongation and attachment, rhizoids were allowed to form on six types of material: agar, gelatin, white petrolatum, silicone grease, plastic, and glass. Semi-circular depressions on soft substrata were made by creating bubbles on melted 2% agar and gelatin (Nacalai Tesque Inc., Kyoto, Japan), or carving using a thin spatula on white petrolatum (Yamazen Pharmaceutical Co., Ltd., Osaka, Japan) and silicone grease for high vacuum (Dow Corning Toray Co. Ltd., Tokyo, Japan). Each type of substratum was 8–10 mm thick, poured after melting or applied to the bottom of glass Petri dishes which were filled with 30 ml PES. Cells were deposited inside the depressions on agar, gelatin, white petrolatum, and silicone grease. In glass Petri dishes and plastic (polystyrene) plates (Iwaki, Tokyo, Japan), cells readily settled in corners between the side and bottom walls of the vessels containing culture medium. Samples were incubated for 14 days in the abovementioned culture conditions, and the number and shape of basal rhizoids in contact with the substratum were recorded.

Effect of light conditions on rhizoid tip growth and cytoplasmic streaming

Elongating rod-shaped rhizoids were observed to change shape after continuous incubation on soft substrata when tips had reached a considerable distance where light is brighter relative to the basal area of the cells. In order to determine the effect of light on elongation of rhizoids, cells were cultured in the dark at different time intervals. Cells in agar slots inside glass Petri dishes were placed in darkness at 22°C for 7, 10, 13, 16, or 19 days. The shape and length of rhizoids formed after each period were recorded. In addition, cells with these elongating rhizoids were taken out of darkness and exposed to 14:10 h LD at 40 μmol photons m^{-2} s^{-1} light sequentially for 14 days and observed for rhizoid shape changes. To determine the range of light intensity that is

effective in inducing shape changes in rhizoids, cells previously incubated in darkness for 14 days were placed at different distances from a white fluorescent light source at 14:10 h LD cycles corresponding to varying levels of light intensity. Measured light intensities were: 2, 10, 15, 35, 72, 103, 200, 305, and 1,020 μmol photons m^{-2} s^{-1}. Tip growth and cytoplasmic streaming were noted in each treatment after 10 days, including the ratio of the widths of peri-apical (swollen) and tubular (no swelling) regions of rhizoids as a measure indicating the degree of swelling. In the case of rod-shaped rhizoids, measurements were taken at 100 and 500 μm from the tip, respectively.

A setup was also made wherein rhizoid-producing cells, previously incubated in agar for 14 days in the dark, were exposed to a 6:3:6-day light/dark/light regime (continuous light, 40 μmol photons m^{-2} s^{-1}). A number of rhizoid cell formations (e.g., dumbbell-shaped cells) were noted.

Staining of rhizoid cell wall

To investigate the cell wall of rhizoids, a fluorescent stain, Fluostain I (Dojin East, Tokyo, Japan), that specifically binds to plant and fungal cell walls (Hughes and McCully 1975) was used. Fluostain I was diluted in PES to a concentration of 0.1 mg/ml and then used to incubate samples for approximately 30 min. Samples were washed three times with PES, placed in the depression of a depression slide filled with PES, covered with a cover slip, and then viewed under an Olympus BX-51 epifluorescence microscope (Olympus Optical Co., Ltd, Tokyo, Japan) with UV.

Effect of photosynthesis inhibitor on bulb-shaped rhizoid formation

To investigate the role of photosynthesis-dependent processes on the observed modification in rhizoid cells, a photosynthesis-inhibiting chemical, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), was utilized. Cells in agar slots inside Petri dishes with PES cultured in darkness for 14 days at 22°C were supplemented with 1 μM and 100 nM DCMU dissolved in dimethylsulfoxide (DMSO) then placed in 14:10 h light/dark at 22°C. Controls were made by preparing 0.005% DMSO/PES (*v*/*v*) and PES only and incubating cells in identical temperature and lighting conditions. DMSO had no effect on the growth and rhizoid formation of cells including the formation of bulb-shaped rhizoids. Rhizoid shapes were recorded after 10 days. Observations on samples were undertaken using an inverted (CKX41, Olympus Optical Co., Ltd, Tokyo, Japan) or a dissecting (SZX7, Olympus Optical Co., Ltd, Tokyo, Japan) microscope attached to a digital camera (Coolpix P6000, Nikon Co., Ltd, Tokyo, Japan) for documentation.

Results and discussion

The initial morphology of rhizoid tips depended on substrata and not on light conditions. Continuous elongation of rod-shaped rhizoids from the basal area of *V. aegagropila* mother cells (Fig. 1a) was promoted by embedding thallus cells in soft substrata such as agar, gelatin, white petrolatum, and silicone grease (Fig. 2a) under LD cycles. In such cases, rhizoids did not exhibit any other mode aside from tip growth and indefinitely increased in length in one general direction. When

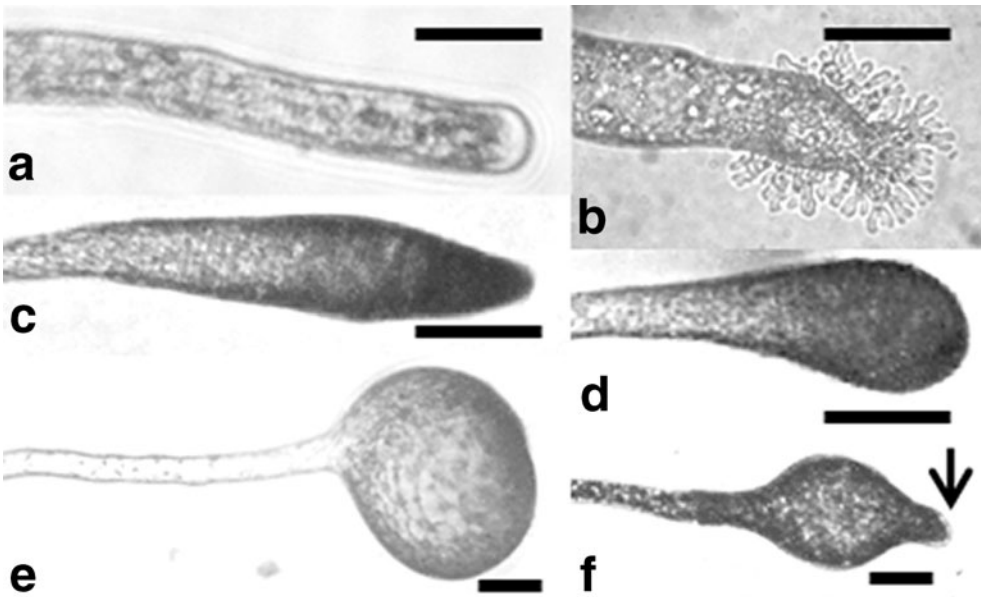
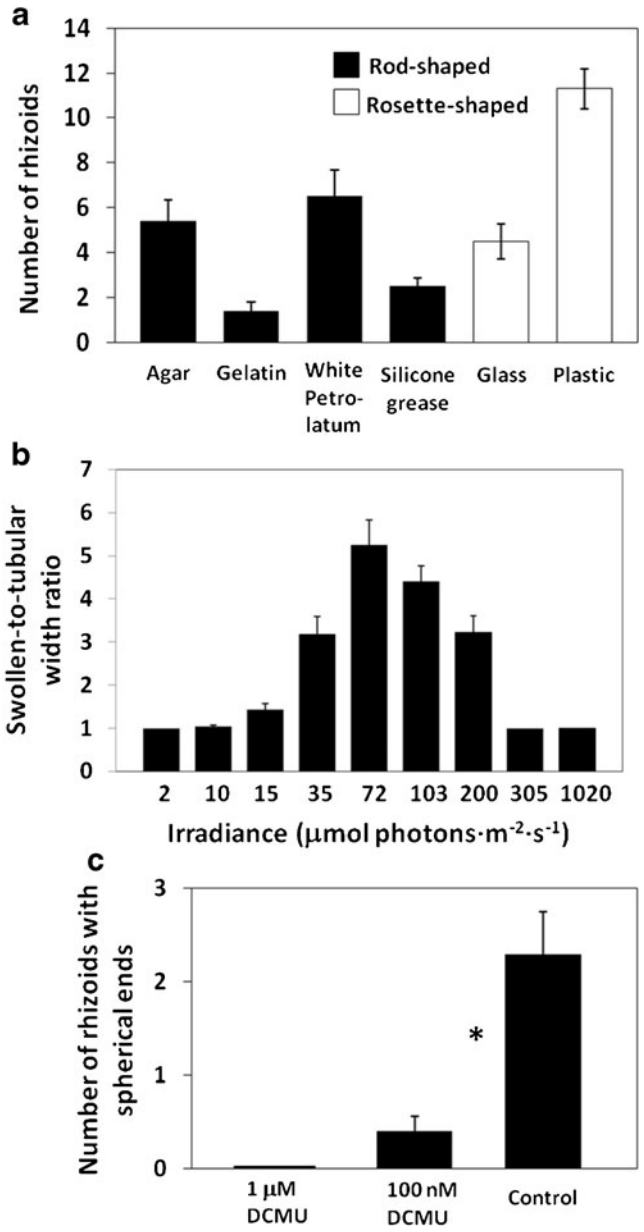


Fig. 1 Growth patterns of *V. aegagropila* rhizoids. **a** An elongating rod-shaped rhizoid incubated in continuous darkness. **b** A rhizoid tip with fine filaments attached on a plastic substratum. **c** A spear-shaped rhizoid resulting from a 72-h continuous exposure to light. **d** A bulb-shaped cell after a 96-h continuous exposure to light. **e** A cell with a spherical end after more than 5 days of exposure to light. **f** A bulb-tipped cell resuming tip growth (*arrow*) after 96 h of continuous light and subsequent 24 h of darkness. *Scale bars* represent 100 μm. Illumination was performed with a white fluorescent lamp (40 μmol photons m^{-2} s^{-1})



◀ **Fig. 2** Effects of substrata, light, and DCMU on rhizoid shape. **a** Types of *V. aegagropila* rhizoids formed on different kinds of materials. Cells were allowed to form rhizoids on six types of substratum (agar, gelatin, white petrolatum, silicone grease, glass, and plastic) under normal culture conditions. Rod-shaped rhizoids continuously elongate on soft substrata while rosette-shaped rhizoids possess finely branched tips firmly attached to hard substrata. Data are mean±SE number of rhizoids directly in contact with the substratum after 14 days (n=10). **b** Effect of light irradiance on the shape of rhizoids. Cells previously incubated in darkness were exposed to increasing light intensity for 10 days. Swelling was measured by dividing the width of swollen region with the width of the tubular (neck) region. In case of rod-shaped rhizoids, measurements were taken at 100 and 500 µm from the tip, respectively. Data are mean±SE number of bulb-tipped rhizoids formed on mother cells (n=10). **c** The effect of a photosynthesis inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (*DCMU*) on spherical tip formation of *V. aegagropila* rhizoids. Cells in 2% agar slots were previously incubated in darkness for 14 days to induce long rod-shaped rhizoids before exposing to 14:10 h LD regime (light, 40 µmol photons m^{-2} s^{-1}) with 100 nM and 1 µM DCMU and without DCMU (negative control). Data are mean±SE number of spherical rhizoid tips formed on mother cells (n=10) after 10 days. *Asterisk* denotes significant difference between treatments (t test, P<0.05)

confronted with hard substrata, under light or dark conditions, tips always flattened and attached to hard substrata through an attachment structure, referred to as a hapteron, without any further obvious growth changes (Fig. 2a and see also below). As such, contact with a plastic substratum induced the construction of a hapteron (Fig. 1b). When the rhizoids attached to hard substrata such as glass or plastic, these rhizoids did not display further diffuse growth nor form other structures.

Conversion between tip growth and diffuse growth was correlated with light conditions. In darkness and on soft substrata, tip growth was continuous which increased the length of a rhizoid. However, upon exposure to white fluorescent lamp light at LD cycles or continuous light regime, dark-incubated rhizoid tips changed shape. Swelling of the cell wall in the apical region occurred after 72 h of continuous light at 40 µmol photons m^{-2} s^{-1}, producing a spear-shaped rhizoid (Fig. 1c). As tip growth was terminated, there was a transformation from a rod-shaped rhizoid into a club-shaped structure (Fig. 1d). Continuous incubation at 40 µmol photons m^{-2} s^{-1} for at least 120 h induced up to 10 rhizoids (n=50) with spherical ends (Fig. 1e). When a rhizoid was maintained at 40 µmol photons m^{-2} s^{-1} continuously for 96 h and then replaced in the dark for at least 24 h, all bulb-shaped structures (n=50) that had formed resumed tip growth (Fig. 1f, arrow).

During plant tip growth, the dome of a cylindrical cell undergoes cell deformation and wall deposition wherein inserted secretory vesicles add new plasma membrane, cell wall precursors, and enzymes at the growth site (Steer and Steer 1989). The cell wall is first loosened and then cellulose microfibrils are deposited parallel to each other resulting in a transverse expansion of the wall. The nascent cell wall around the dome is thin and pliable while the cell wall next to the apical dome increases in thickness and strength as distance from the apex increases (Kropf et al. 1998). Thus, expansion is localized at the tip region where wall loosening and deposition occur, whereas in the other parts wall strength is maintained. In diffuse growth, extensibility of the wall is not localized but extends all over the cell and is achieved through cell elongation perpendicular to the orientation of microfibrils (Martin et al. 2001). Cell wall loosening agents, such as expansin, have been suggested to facilitate deformation of the wall before the deposition of a new wall (Cosgrove 2000).

When the cell walls of rhizoids were stained with Fluostain I, rhizoids that were incubated in the dark then exposed to light had a spear shape exhibiting wall striations near the

tip where swelling occurred (Fig. 3a, arrow). The occurrence of such striations was associated with decreased intensity of staining on the swollen area. On the other hand, at either ends of the swollen part, the cell wall was continuous, displayed no striations, and had moderate staining (Fig. 3a, arrowheads). The tip had stopped growing at this point, thus loosening of the cell wall that permits tip expansion could have been terminated also. The moderate staining at the tip may indicate the presence of some amounts of new cell wall materials as both cell wall loosening and wall deposition ceased. Cell wall fluorescent staining in fungi clearly revealed an increased wall deposition at the extreme tip regions of growing hyphae (Gull and Trinci 1974). Wall loosening in *Valonia* seemed to have shifted or translocated from the extreme tip to the sub-apical area where swelling or any sign of diffuse growth had never before occurred. The less intense staining in this swollen area may indicate lower wall deposition activity coupled with wall loosening. When the cell wall of a bulb-shaped rhizoid, placed in the dark to induce resumption of tip growth, was stained with Fluostain I the tip showed increased staining (Fig. 3b, arrowhead). The round part, on the other hand, displayed less intense staining even when compared to that of the tubular part of the rhizoid where the cell wall had thickened through time. At this point, diffuse growth along the round region had stopped. It appears that wall loosening and wall deposition had returned to the tip. Thus, the switches in growth mode likely involved alternating translocation of wall loosening from the tip to the sub-apical region and then back to the tip. A review of available literature on location-based coordination in cell wall loosening and deposition did not reveal descriptions fitting our present observations.

Cytoplasmic streaming was not affected during change in growth mode. Light intensities in the range of 2–200 μmol photons m^{-2} s^{-1} did not inhibit cytoplasmic streaming in rhizoids. However, continuous cytoplasmic streaming and expansion at the rhizoid tip at 15–200 μmol photons m^{-2} s^{-1}, resulted in the bulging of rhizoid tips (Fig. 2b). At 305 and 1,020 μmol photons m^{-2} s^{-1}, cytoplasmic streaming was inhibited due to extreme light intensity wherein cells might have been damaged. Cytoplasmic streaming has been reported in several giant algal cells (Kamiya 1986). In *Acetabularia*, extended incubation in darkness arrested cytoplasmic streaming but recovered after illumination (Dazy et al. 1989). In this study, acropetal cytoplasmic streaming occurred at 15 to 200 μmol photons m^{-2} s^{-1}, filling the intracellular space as the cell wall loosened and expanded (Online Resource 1).

Changes in the rhizoid morphology involved light-dependent, energy-requiring processes. This was confirmed by the introduction of a photosynthesis inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea. DCMU blocks the electron transfer between the primary and secondary receptors on the reducing side of photosystem II (Metz et al. 1986). A relatively low concentration of DCMU (100 nM) allowed formation of a small number of bulb-shaped rhizoids but diffuse growth was totally inhibited at 1 μM DCMU (Fig. 2c). Rhizoids treated with 1 μM DCMU continued to elongate but did not show any sign of continuous diffuse growth in the tip region, characteristic of spherical rhizoids that eventually enlarged when maintained in illuminated environment. When rhizoid cells were exposed to a 6:3:6-day light/dark/light regime at 40 μmol photons m^{-2} s^{-1}, up to 12 rhizoid ends (n=50) formed a secondary swollen tip, forming a dumbbell-shaped structure still capable of tip growth when finally placed in the dark for 24 h (Fig. 4a, arrow). These rotund cells with terminated tip growth irreversibly increased in size after continuous lighted incubation, and resembled lateral branch cells (Fig. 4b). Although underground plant organs such as roots are heterotrophic, these have been shown to be capable of facilitating photosynthesis when exposed to light (Flores et al. 1993). However, algal rhizoids are basically substratum-attachment structures that form on darkly illuminated regions of thalli

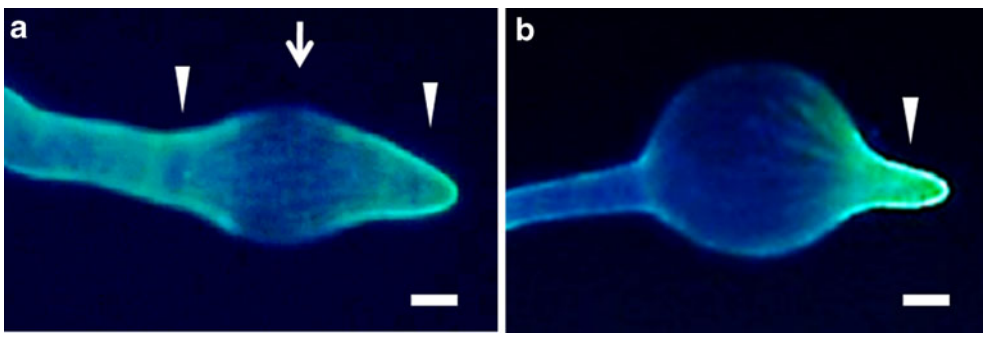


Fig. 3 Fluostain I-stained rhizoid cell walls. **a** A spear-shaped rhizoid incubated in the dark for 14 days and then exposed to 40 μmol photons m^{-2} s^{-1} for 2 days showing striations on the cell wall of swollen region (*arrow*) while the tip and non-swelling regions had continuous and bright staining (*arrowheads*). **b** Resumption of tip growth in a bulb-tipped rhizoid after incubating in darkness for 14 days and subsequently exposing to 40 μmol photons m^{-2} s^{-1} for 6 days and then placing in the dark for 3 days, showing intense staining at the tip growing region (*arrowhead*). *Scale bars* represent 20 μm

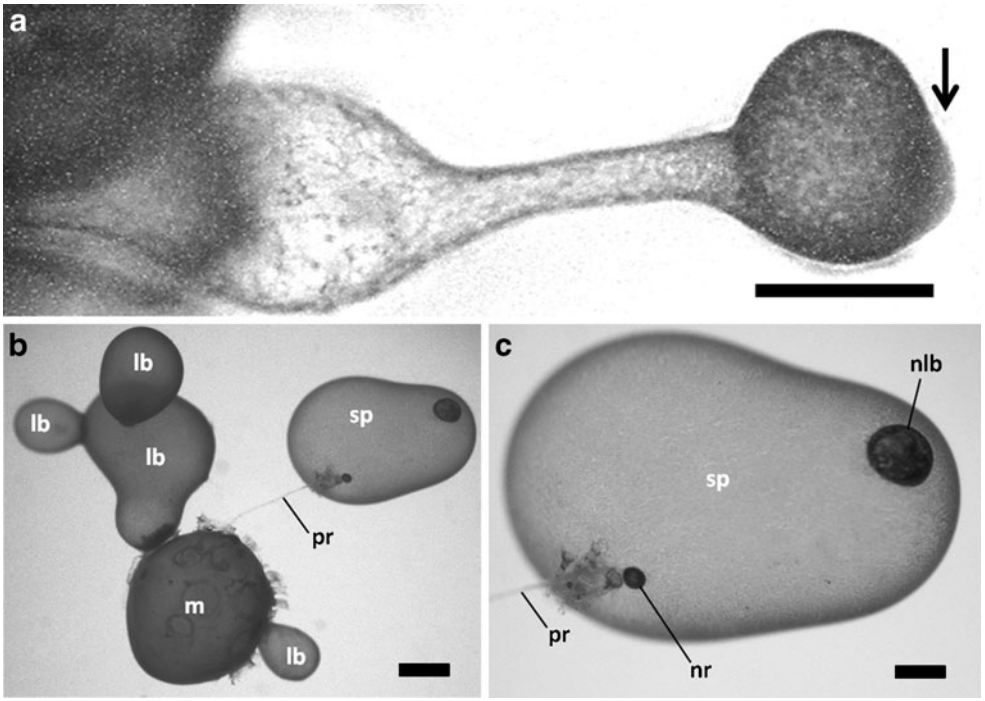


Fig. 4 Formation of a secondary spherical tip, lateral branch cells, and new rhizoids. **a** An aseptate dumbbell-shaped cell formed after a 6:3:6-day light/dark/light regime resuming tip growth (*arrow*) after finally placed in darkness for 24 h. **b** A sample showing the mother cell (*m*), lateral branch cells (*lb*), the primary rhizoid tubular part (*pr*), and the spherical/rotund part (*sp*) after approximately 8 months of exposure to 14:10 h LD cycles. **c** the spherical/rotund part of the primary rhizoid from **b**, showing lenticular cells of a new rhizoid (*nr*) and a new lateral branch cell (*nlb*). *Scale bars*: **a**, **b** 100 μm, **c** 50 μm. Cells were incubated under white fluorescent light (40 μmol photons m^{-2} s^{-1})

(Fritsch 1935; Bold and Wynne 1985) and these results indicate that the conversion from rod-shaped rhizoids to spherical rhizoids requires energy-generating processes including photosynthesis.

After several months of continuous incubation on agar and LD cycles, large, spherical parts of rhizoids started producing lateral branches (new thallus cells) of their own through lenticular cell formation (Fig. 4c). This indicates that rhizoids may transform into thallus cells, too. In the presence of light with definite ranges of intensity, rhizoids stopped tip growth and exhibited expansive (diffuse) growth at the tip regions to become spherical similar to thallus cells. The rhizoids having spherical parts can restart tip growth at the opposite ends after they were placed in the dark. This means that the rhizoids remember the ability of tip growth that they originally had. Although little is known regarding this cellular phenomenon, it could indicate light-induced inhibition of expression of genes such as those found in the rhizoid-forming moss *Physcomitrella patens* (see below). However, if the spherical parts completely convert into thallus cells, they should carry out lenticular cell formation. In *Valonia*, rhizoids start from small lenticular cells at the basal part of mother cells, whereas thallus cells develop from large lenticular cells at the lateral part of mother cells (Bold and Wynne 1985; Okuda et al. 1997). The fate of these two kinds of cell is determined when lenticular cell formation occurs, depending on the position and size of a lenticular cell produced. Thallus cells undergo diffuse growth while rhizoid cells exhibit tip growth. The present study demonstrated that rhizoid cells differentiate into thallus cells directly without lenticular cell formation. When such thallus cells developed from rhizoid tips produce new thallus branch cells and rhizoids, they have to cleave lenticular cells (Fig. 4c) from which thallus cells and rhizoids elongate. There might be some threshold where rhizoid cells differentiate into thallus cells or not. Once thallus cells are fully differentiated, they can produce both new thallus cells and rhizoid cells but only through lenticular cell formation (cell divisions; Fig. 4c). Swelling of rhizoid tips induced by light exposure may indicate a transitional stage toward the differentiation of thallus cells from rhizoids. If this is the case, rod-shaped rhizoids are regarded as nascent or immature cells rather than fully differentiated cells, unlike thallus cells. The primary function of rhizoids is to adhere to substrata by forming rosette-like haptera. Some of the rhizoids that do not find any suitable substrata may stop elongation in bright places and bulge at their tip to develop new thallus cells (Fig. 5). Thus, the potential to transition from tip growth to diffuse growth in rhizoids may serve as one of the vegetative propagation modes in *Valonia*. In *Arabidopsis*, Sugimoto et al. (2010) have shown the resemblance between the behaviors of the root tip meristem and callus tissue, wherein each has the ability to grow aerial shoots. Such a type of redifferentiation in plants can also be induced by stress-responsive genes, causing cells to dedifferentiate before assuming a new fate (Grafi et al. 2011). In algae, a mechanical stress such as wounding has been shown to cause thallus-to-rhizoid redifferentiation in *Griffithsia pacifica* (Waaland and Watson 1980) and *Spirogyra* (Inoue et al. 2002). In terms of development, relevant genes for rhizoid differentiation have been discovered in some

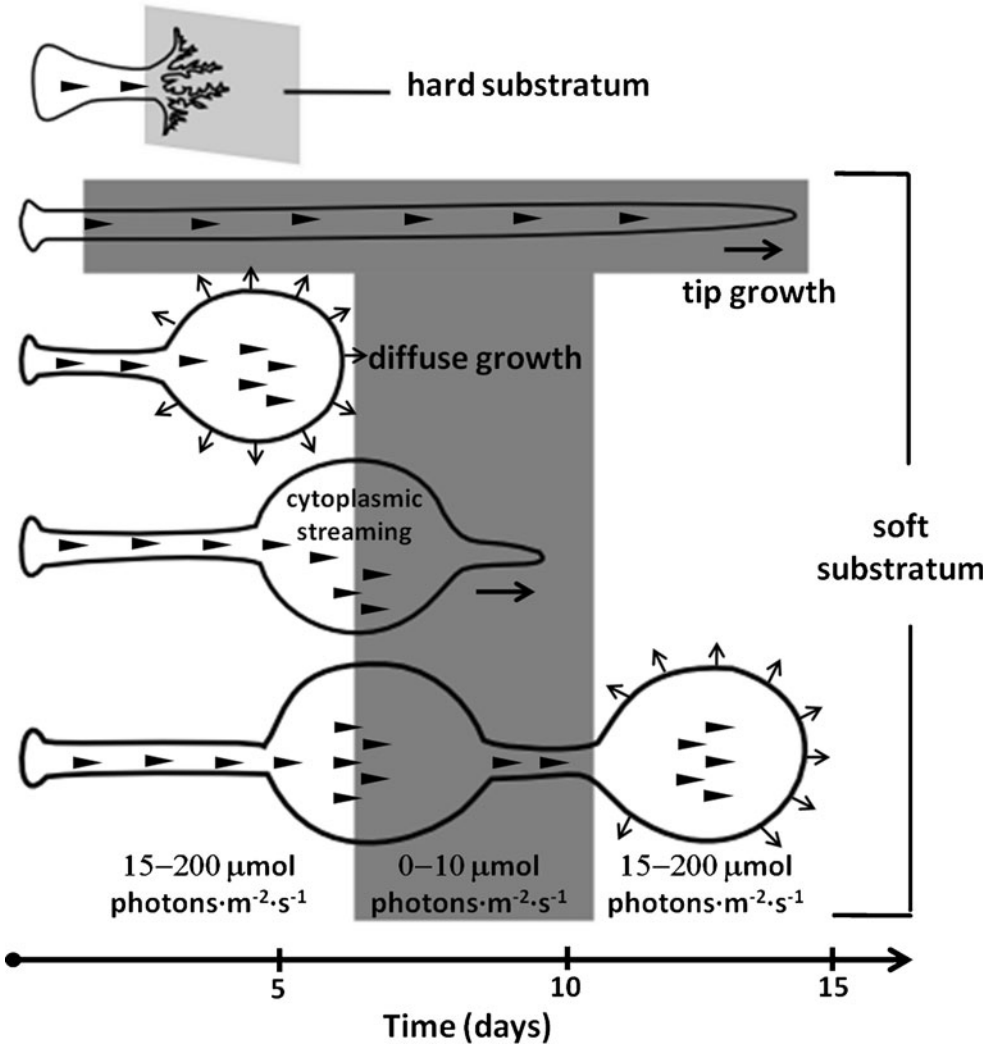


Fig. 5 Diagrammatic representation of growth patterns of *V. aegagropila* rhizoids in response to substrata and light conditions through time. When a rhizoid makes contact with a hard substratum such as glass, the tip flattens and produces filaments comprising the attachment structure called a hapteron, under illuminated or dark conditions. Without substratum and on soft substrata such as agar, low light intensities (less than 10 µmol photons m^{-2} s^{-1}) promote rhizoid elongation through continuous tip growth. Under high light intensities (15–200 µmol photons m^{-2} s^{-1}) and on a soft or without substratum for approximately 5 days, tip growth in rod-shaped rhizoids stops while cytoplasmic streaming is continuous concomitant with the formation of a spherical tip. When a rhizoid with a round tip is placed again under dark or low light conditions for approximately 3 days, tip growth resumes. Finally, when a rhizoid with a recovered tip growth is returned under a bright light environment, a secondary spherical shape is produced at the tip. *Arrows* the direction of growth, *arrowheads* the direction of cytoplasmic streaming

model species. For instance in *P. patens*, phosphatidylinositol phosphate kinases, responsible for the synthesis of the lipid messenger PtdIns-4,5-biphosphate, have been found to be critical during rhizoid elongation and caulonemal cell formation (Saavedra et al. 2011) while *P. patens* RHD-SIX-LIKE1 (PpRSL1) and PpRSL2 have been shown to convert gametophores into rhizoids (Jang et al. 2011). In addition, an auxin-inducible homeodomain-leucine zipper I gene, *Pphb7*, has been reported to be involved in *P. patens* epidermal cell differentiation into a rhizoid (Sakakibara et al. 2003).

Mosses are one of the most ancient lineage of plants that react to phytohormones such as auxin (Johri 2008). Farther back is *Chara*, a green alga and one of the closest algal relatives of the land plants, forming rhizoids that also depend on auxin (Klambt et al. 1992). Analogous auxin functions in other green algae such as *Bryopsis* (Jacobs 1951) and *Caulerpa* (Jacobs et al. 1985) have been likewise reported. Auxin also regulates rhizoid formation during embryogenesis of the brown alga *Fucus* (Basu et al. 2002). Finding a comparable mechanism that connects light regimes and auxin response in *Valonia* is highly likely. This might also lend further credence to the suggestion that auxin-regulated rhizoid formation in *Chara*, *Fucus*, and other algae is an ancient developmental response in photosynthetic aquatic organisms (Cooke et al. 2002).

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Conflicts of interest The authors declare that they have no conflict of interest.

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