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Diffuse cell elongation in two bangiophyte red algae: *Rhodochaete parvula* and *Porphyra yezoensis*

David J. Garbary* and Francis Magne**

*Department of Biology, St. Francis Xavier University, Antigonish, Nova Scotia, B2G 1C0, Canada
**Laboratoire de Biologie Végétale Marine, Université Pierre & Marie Curie (VI), 7 Quai Saint-Bernard, 75230 Paris, France

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Calcofluor stained plants of *Rhodochaete parvula* Thuret and conchocelis of *Porphyra yezoensis* Ueda showed tip growth in apical cells and diffuse growth in intercalary cells. Over the 4–8 day experimental period 3–7 new cells were added at each branch apex with subapical cells expanding about 40% and 70% in length, respectively, with no increase in cell diameter. Diffuse elongation is considered the primitive method of intercalary cell wall deposition in apically growing red algae, whereas band deposition, previously observed in Batrachospermales and Ceramiales, is considered the derived condition.

Key Index Words: Bangiophycidae—calcofluor—cell walls—cell elongation—cell wall deposition—Porphyra—Rhodochaete—*Rhodophyta*.

Waaland *et al.* (1972), Waaland and Waaland (1975) and Aghajanian and Hommersand (1980) demonstrated that red algae have tip growth in apical cells and band deposition in intercalary cells. Hymes and Cole (1983), however, were able to find only tip growth in the freshwater red alga *Audouinella hermannii* (Roth) Duby. More recently, diffuse cell wall deposition was demonstrated for the first time in red algae in three species of Florideophycidae in the genera *Audouinella* (Acrochaetiales), *Spermothamnion* and *Tiffaniella* (Ceramiales) (Garbary and Belliveau 1990). The occurrence of diffuse growth in the potentially primitive order of florideophyte red algae (i.e. Acrochaetiales) (Gabrielson and Garbary 1987, Garbary and Gabrielson 1987) suggests that this is the plesiomorphic or primitive character state for the subclass Florideophycidae.

Rhodochaete and conchocelis of *Porphyra* (or *Bangia*) are particularly appropriate organisms for studying the evolution of cell wall deposition characters in Rhodophyta, and establishing the primitive character state for florideophyte red algae. With their filamentous construction, apical cell division and presence of plugged pit connections, basic thallus morphology in these genera is homologous to that in florideophyte red algae (Garbary and Gabrielson 1990). Thus mechanisms of cell wall deposition are likely to provide homologous features for comparison. If diffuse elongation is present (in the absence of band deposition) in Rhodochaetales, Bangiales, and Acrochaetiales, it can be concluded that diffuse growth is the primitive mechanism of intercalary cell wall deposition in apically growing red algae.

Materials and Methods

Rhodochaete parvula was isolated from the Mediterranean Sea and cultured in enriched seawater media as described by Pueschel and Magne (1987). Conchocelis of *Porphyra yezoensis* (U-51) from Japan was grown in a modified von Stosch medium (Guiry and Cunningham 1984). Plants were stained with 0.01% or 0.001% calcofluor white (Fluorescent Brightener 28 from Sigma) for 30 min, as described by Garbary and Belliveau (1990),

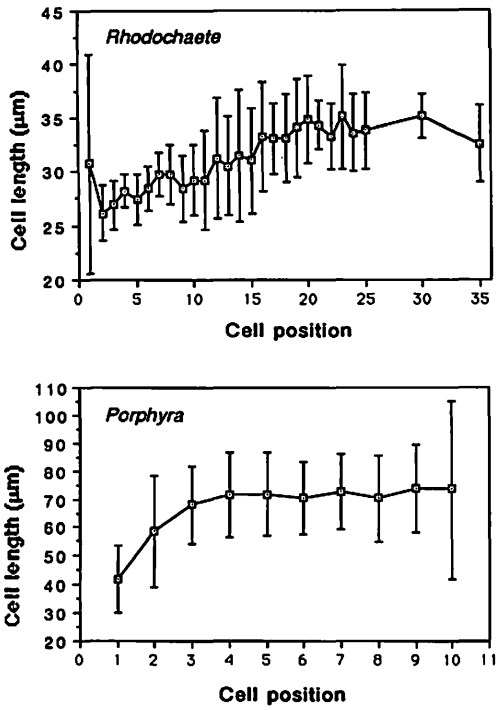

Fig. 1. Patterns of cell elongation along primary axes of *Rhodochaete parvula* and conchocelis of *Porphyra yezoensis*. Values indicate means±s.d. (n=12).

and grown for four (*Porphyra*) or eight days (*Rhodochaete*) in calcofluor-free medium. It should be noted that longer exposures or higher concentrations of calcofluor may cause reductions in growth or growth abnormalities in red algae (Belliveau *et al.* 1990).

Fluorescence microscopy and microspectrofluorometry were carried out on a Zeiss Photomicroscope III using Zeiss accessories as described in Garbary and Belliveau (1990). Measures of cell wall (calcofluor) fluorescence from 5-10 different cells were made from the middle portions of cells of *Porphyra* at cell positions 1, 3, 5, 7, 8 using a 3 µm diameter aperture. Similar measurements on *Rhodochaete* were not carried out because of high background fluorescence.

Morphometric analysis of cell length was carried out by measuring the lengths of cells 1-25, 30 and 35 in *Rhodochaete* (cell 1 is the apical cell), and cells 1-10 in conchocelis of *Porphyra*. In each case twelve filaments were measured.

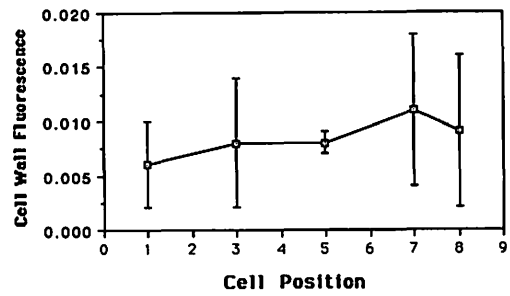

Fig. 2. Change in relative fluorescence along a filament of *Porphyra*. Note: Cell 1 would have been the subapical cell at time of staining; when material was fixed this was cell 5-8. Values indicate means±s.d. (n=5-10).

Results

Calcofluor at 0.01% and 0.001% was apparently non-toxic for *Porphyra* and *Rhodochaete*, and apical cell division proceeded at 0.5-1 cell divisions per day after staining. Cell length measurements along unstained primary axes of *Rhodochaete* and *Porphyra* show that some cell elongation is occurring in intercalary cells (Fig. 1). The average total elongation (as a % of subapical cell length) is about 40% in *Rhodochaete* and 70% in *Porphyra*. In *Rhodochaete* there is a gradual elongation to about cell 16 after which cell length remains stable. In *Porphyra* cell length increases are basically limited to cells 2-4 although occasional much larger (or smaller) cells are found in older portions of filaments (note much larger s.d. at position 10).

Growth experiments using calcofluor show that no band elongation is occurring. Microspectrofluorometry of calcofluor stained cells of *Porphyra* showed a possible increase in fluorescence away from what were previously apical cells after the 4 day growth period (Fig. 2). The slightly lower fluorescence values closer to the old apex (cells one to five) may have resulted from greater wall deposition in these cells (after staining) than cells that were more fully elongated.

Discussion

Early work by Waaland and Waaland

(1975) and Aghajanian and Hommersand (1980) suggested that band elongation was the primary method of intercalary cell wall deposition in Rhodophyta, although a later study by Hymes and Cole (1983) did not show the presence of band growth in *Audouinella hermannii*. Several other *Audouinella* species have been shown to have diffuse cell wall deposition: *A. dasyae* (Garbary and Belliveau 1990), *A. botryocarpa* and *A. pacifica* (Garbary and Guiry, unpublished). That species of *Rhodochaete, Porphyra* and *Audouinella* all have diffuse growth in intercalary cells suggests that this is the primitive condition for florideophyte red algae. Although we have not examined other angiophytes in either Compsopogonales (e.g. Erythropeltidaceae) or Porphyridiales, the small cell sizes and the patterns of thallus construction in these algae are not suitable for band elongation.

We have demonstrated that intercalary cells of both *Rhodochaete* and *Porphyra* have cell elongation. The amount of cell elongation, however, is among the smallest recorded for apically growing red algae. The absence of band deposition in elongating intercalary cells suggests that cell elongation is by diffuse growth.

The occurrence of diffuse cell wall deposition in three paraphyletic orders near the base of the red algal phylogenetic tree (Gabrielson *et al.* 1985, Gabrielson and Garbary 1987) leads to the conclusion that this is the primitive character state for florideophytes, with band elongation being the more advanced condition. Several evolutionary scenarios (Fig. 3) can be suggested from our results. In the first scheme (Fig. 3A) unicellular or multicellular morphology is considered primitive, such organisms having only diffuse cell wall deposition. With the evolution of apical cells, tip growth became possible, along with the filamentous morphologies of *Rhodochaete*, conchocelis of *Porphyra* and florideophytes. Some florideophyte red algae then evolved band deposition. The question remains whether band deposition arose once, or more than once, in separate florideophyte lineages.

A second scheme (Fig. 3B) places an organism similar to *Rhodochaete* in an ancestral position. This organism may have had only tip growth or a combination of tip growth and diffuse elongation. Some of the descendants of this organism lost the ability to form apical cells, and cell wall deposition became exclusively diffuse. As florideophytes evolved, one or more evolutionary lines then developed band elongation. The absence of band elongation in some Ceramiaceae is considered an evolutionary loss in a highly derived group of Ceramiaceae (Garbary and Belliveau 1990, Hommersand 1990). This scheme is based on the notion that *Rhodochaete*, as the archetypal red alga, is the most primitive extant member of the division. Yet another evolutionary scheme was presented by Magne

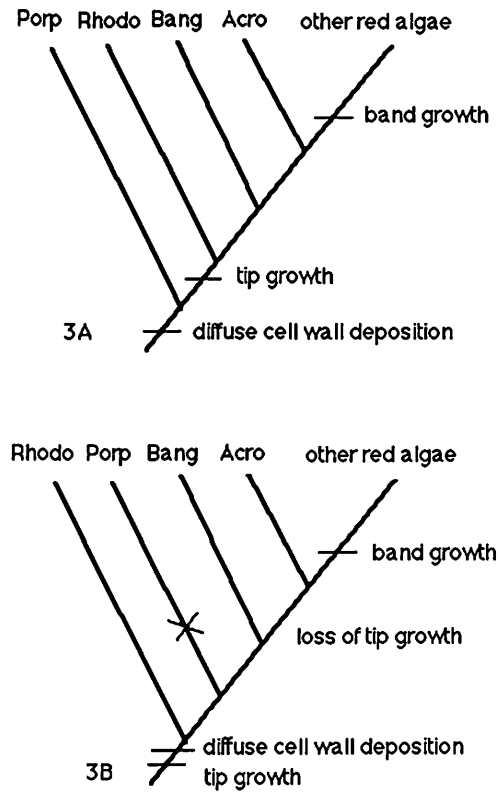

Fig. 3. Phylogenetic schemes based on the evolution of different cell wall deposition patterns. 3A-Diffuse wall deposition primitive; 3B-Tip elongation or tip and diffuse elongation primitive. See text for discussion. Abbreviations: Porp—Porphyridiales; Rhodo—Rhodochaetales; Bang—Bangiales; Acro—Acrochaetiales.

(1989) in which two evolutionary lines (subclasses) of red algae "Eurhodophycidae" and Metarhodophycidae" evolved independently from the "Archaeorhodophycidae". This scheme would require that tip growth was independently derived in *Porphyra* and *Rhodochaete*. These three evolutionary hypotheses become testable with the application of DNA and/or RNA sequencing.

Although bangiophytes are apparently consistent in terms of cell wall deposition patterns, having either diffuse or diffuse and tip growth, preliminary studies show that variation in this feature among florideophytes (Garbary and Belliveau 1990) has considerable potential as a phylogenetic feature at ordinal, family and tribal levels. Further studies of cell wall deposition patterns in red algae are clearly in order.

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D. J. Garbary*·F. Magne: 原始紅藻2種 (*Rhodochaete parvula, Porphyra yezoensis*) における分散細胞伸長**

カルコフルオール染色した *Rhodochaete parvula* 藻体とスサビノリ *Porphyra yezoensis* の糸状体は，頂端細胞では頂端成長を，介在細胞では分散成長を示した。4－8日の実験期間中に，各側枝先端で3－7細胞が新しく付け加わり，長さはそれぞれ約40%および70%増加したが，細胞の直径は増大しなかった。分散伸長は頂端成長を行なう紅藻における介在細胞壁の原始的な沈着法であり，カワモズク目とイギス目ですでに観察されている帯状沈着は派生的なものと考えられる。(*Department of Biology, St. Francis Xavier University, Antigonish, Nova Scotia, B2G 1C0, Canada; **Laboratoire de Biologie Végétale Marine, Université Pierre & Marie Curie (VI), 7 Quai Saint-Bernard, 75230 Paris, France)