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Mitochondrial Dynamics in Red Algae. 3. Filament Apices in *Colaconema caespitosum* (Acrochaetiales) and *Antithamnion cruciatum* (Ceramiales)

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Mitochondrial distribution and abundance were assessed during the growth of apical and subapical cells in the red algae *Colaconema caespitosum* (J. Agardh) Jackelman, Stegenga and Bolton and *Antithamnion cruciatum* (C. Agardh) Nägeli after staining with 3,3'-dihexyloxacarbocyanine iodide [$DiOC_6(3)$] and 2,4'-dimethylaminostyryl-N-ethylpyridinium iodide (DASPEI). In fully elongate apical cells of *C. caespitosum* there were 100-120 mitochondria. During apical cell enlargement and division there is a doubling and then halving of the mitochondrial numbers. Apical cells prior to cytokinesis in young filaments are smaller than in mature filaments (ca. 50 and 100 μm long, respectively) and have fewer mitochondria (ca. 100 and 120 mitochondria per cell, respectively). In older vegetative cells mitochondria tend to aggregate at opposite ends of the cells with some mitochondria associated with the central nucleus or at points of apparent branch initiation. There is a greater density of mitochondria in apical cells of smaller versus larger plants (one mitochondrion per 6.3 μm^3 and 9.8 μm^3, respectively), suggesting that apical cells of younger plants may be more metabolically active. Male and female gametophytic thalli of *Antithamnion cruciatum* had similar numbers of mitochondria in apical cells of indeterminate axes, as did gametophytic and sporophytic thalli. There were about 40-50 mitochondria in fully elongated apical cells with about half this number in newly divided apical and subapical cells. Apical cells of determinate branches had more mitochondria (60-77) than indeterminate branches (60-70 vs. 40-50). In both species and in all cell types mitochondrial numbers were highly correlated with cell size.

Key Words: Acrochaetiaceae, *Antithamnion*, apical cells, *Colaconema*, $DiOC_6(3)$, DASPEI, cell elongation, DAPI, mitochondria, Rhodophyta

INTRODUCTION

Although mitochondria are pointed out in virtually all ultrastructural studies of red algae, details of the distribution and abundance of this organelle are limited in Rhodophyta. Unicellular red algae appear to a have single, large and highly convoluted mitochondrion as first demonstrated in the three dimensional reconstruction of *Rhodella maculata* L. Evans (Broadwater and Scott 1986) and later confirmed, based on fluorescence microscopy of *Cyanidium caldarium* (Tilden) Geitler and *Galdieria sulphuraria* (Galdieri) Merola (Suzuki *et al*. 1994). These unicellular red algae have proven to be useful model systems for studies of mitochondrial division (e.g., Kuroiwa 1998, 2000; Miyagishima *et al*. 2001; Misumi *et al*. 2005). Studies of multicellular red algae showed that many mitochondria were present in these algae (Russell *et al*. 1993; Garbary and McDonald 1998; Garbary and Pei 2000a, b). This confirmed ultrastructural studies in which multiple mitochondrial profiles were present in sections (e.g., Pueschel and Cole 1985; Broadwater *et al*. 1986a, b; Delivopoulos and Diannelidis 1991a, b; Schnepf 1992). Mitochondrial associations have also been demonstrated with dictyosomes and nuclei (Pueschel 1990; Scott and Broadwater 1990). Changes in abundance of mitochondria, as well as specific cell localizations, suggest particular metabolic roles that may change with development.

Garbary and Pei (2000a, b) demonstrated that mitochondria can be dynamic organelles in *Colaconema caespitosum* (J. Agardh) Jackelman *et al*. (1991) [as *Audouinella botryocarpa* (Harvey) Woelkerling] with major changes in localization occurring during spore germination and monosporogenesis (Garbary and Pei 2000 a, b). In this paper we focus on mitochondria in apical and subapical cells of the red algae, *C. caespitosum* and *Antithamnion cruciatum* (C. Agardh) Nägeli. We examine mitochondrial dynamics associated with differences in developmen-

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tal state in *C. caespitosum* (small and vegetative versus large and reproductive thalli) as well as the apical systems of determinate and indeterminate axes of *Antithamnion cruciatum*. Based on these observations we can evaluate patterns of cell growth and differentiation, and demonstrate quantitative changes in mitochondrial abundance during the cell cycle.

MATERIALS AND METHODS

Source of material and culture methods

Colaconema caespitosum was isolated from Finavarra, County Clare (Ireland) in August 1986 by M. D. Guiry. See Guiry *et al*. (1987) for a detailed account of the morphology and reproduction of this isolate (as *Audouinella botryocarpa*). Although tetrasporangia have been described in this species, our isolate only produced monosporangia during this investigation. *Antithamnion cruciatum* (C. Agardh) Nägeli was collected from Monks Head Beach, St. Georges Bay, Antigonish Co., Nova Scotia on August 1992. Vegetative and reproductive morphology are described in Maggs and Hommersand (1993). Portions of a vegetative plant were excised and grown in VS5 medium (Guiry and Cunningham 1984). All plants were grown at 13-15°C under continuous light at a photon fluence of 10 μmol m^{-2} s^{-1} provided by cool-white fluorescent tubes.

Pretreatment and staining procedure

Seawater was evaporated at 80-95°C until it reached 25-30% of its original volume. *C. caespitosum* (but not *A. cruciatum*) required immersion in this solution for 6-12 h prior to staining with $DiOC_6(3)$ (3,3′-dihexyloxacarbocyanine iodide) (Sigma D-3652; www.sigmaaldrich.com) or DASPEI (2,4′-dimethylaminostyryl-N-ethylpyridinium iodide) (ICN-207049, ICN Biomedicals, St. Laurent, Quebec, Canada). *A. cruciatum* (but not *C. caespitosum*) was pretreated with 1-5 μL mL^{-1} of b-glucuronidase (Sigma G-7017) for 8-24 hours or 0.01 μL mL^{-1} for one week (similar to Garbary *et al*. 1992, McDonald *et al*. 1993) prior to staining with $DiOC_6(3)$, or DASPEI. Longer enzyme treatments at lower concentration generally gave more satisfactory results. $DiOC_6(3)$ and DASPEI were prepared as described previously (Garbary and Pei 2000 a, b). For staining, plants were immersed in the working solutions of mitochondrial stains for 5-15 min (for *A. cruciatum*) or 5-24 h (for *C. caespitosum*) at room temperature, rinsed briefly with culture medium and then mounted in Citifluor PBS (Marivac Ltd., Halifax, Nova Scotia). No differences in mitochondrial labeling were noted with either DASPEI or $DiOC_6(3)$. Nuclei were stained with DAPI (4′,6-diamidino-2-phenolindole) in the Citifluor PBS mounting medium (Garbary and Pei 2000a, b). The DAPI stock solution was mixed with McIlvaine's buffer (at pH 4.2) (1:9) as described by Goff and Coleman (1984) and then added to the Citifluor preparation (3:1).

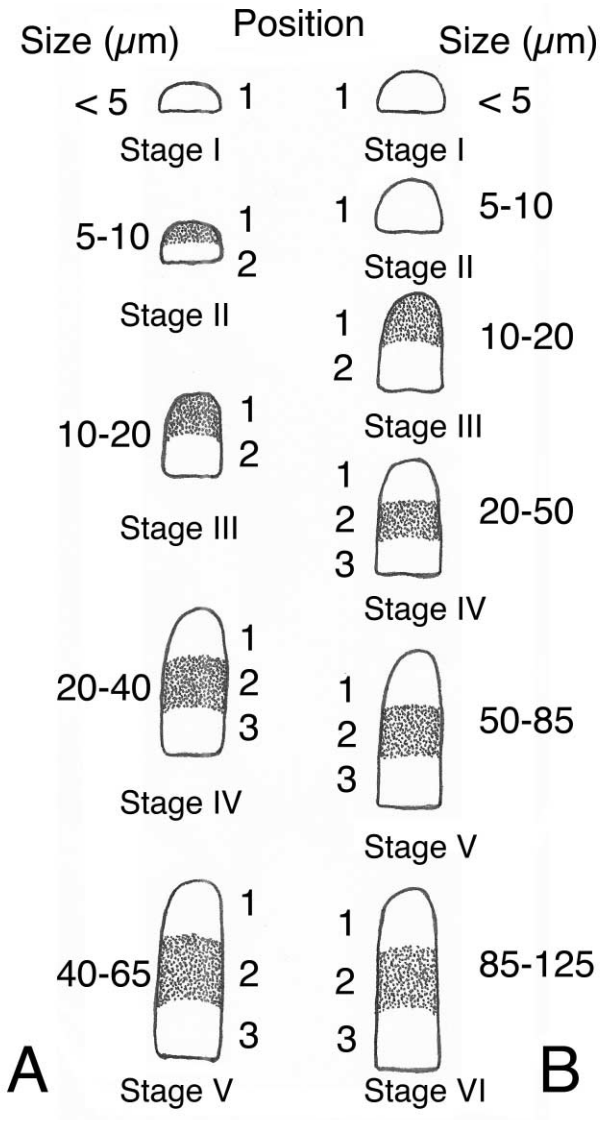


Fig. 1. Schematic of apical cells of *Colaconema caespitosum* at different stages of development described for mitochondrial distribution and abundance. Stippled areas are arbitrary divisions of cells designated for counting.

Fluorescence microscopy and photomicroscopy were carried out using a Zeiss Photomicroscope III as described previously (Garbary and Pei 2000a, b).

Quantification of mitochondria

For quantification, mitochondria were considered

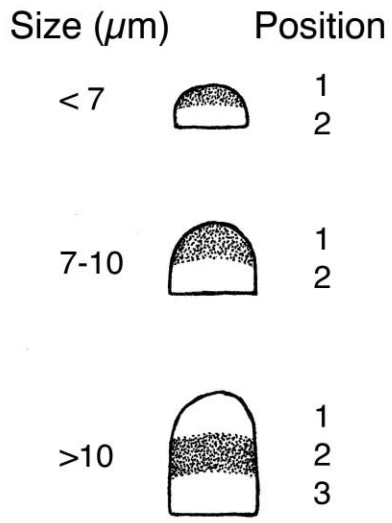


Fig. 2. Schematic of apical cells of *Antithamnion cruciatum* at different stages of development described for mitochondrial distribution and abundance. Stippled areas are arbitrary divisions of cells designated for counting.

either as 'single' or as 'complexes' similar to Garbary and Pei (2000a, b). Single mitochondria were defined as the smallest fluorescing units that could be resolved as mitochondria; larger mitochondrial structures were defined as complexes and counted separately. Mitochondrial distribution in cells was quantified by arbitrarily considering cells at different sizes. Sizes classes ranged from newly divided cells (< 10 μm long) to fully elongated cells (up to 125 μm). In *Colaconema*, four or five stages were distinguished in immature (apical cells 5-65 μm long) and mature filaments (apical cells 5-125 μm long), i.e., in small non-reproductive thalli versus those in larger, reproductive thalli (Fig. 1). Within most of these cells mitochondria were counted in the upper, middle and bottom third of each cell. In some cells in the smallest size category (i.e., ca. 5 μm), mitochondria were not counted because of their density.

The smaller apical cells of *A. cruciatum* (ca. 5-15 μm) were considered in only three size categories (Fig. 2). Mitochondria were counted separately both in the upper and lower (small apical cells) and in the upper, middle and lower parts of apical cells (larger apical cells). *A. cruciatum* has determinate and indeterminate vegetative axes. Mitochondria were quantified separately in both branch types. In determinate branches, mitochondrial counts were also made in subapical cells 2-5.

In most preparations for both species, mitochondria could be quantified before photobleaching precluded completion of counting. Where photobleaching was more rapid (despite use of Citifluor PBS), counts could be made only on parts of cells. Mitochondrial abundance was determined in a minimum of 15 apical cells at each stage of development.

RESULTS

Mitochondria in apical cells of *Colaconema caespitosum*

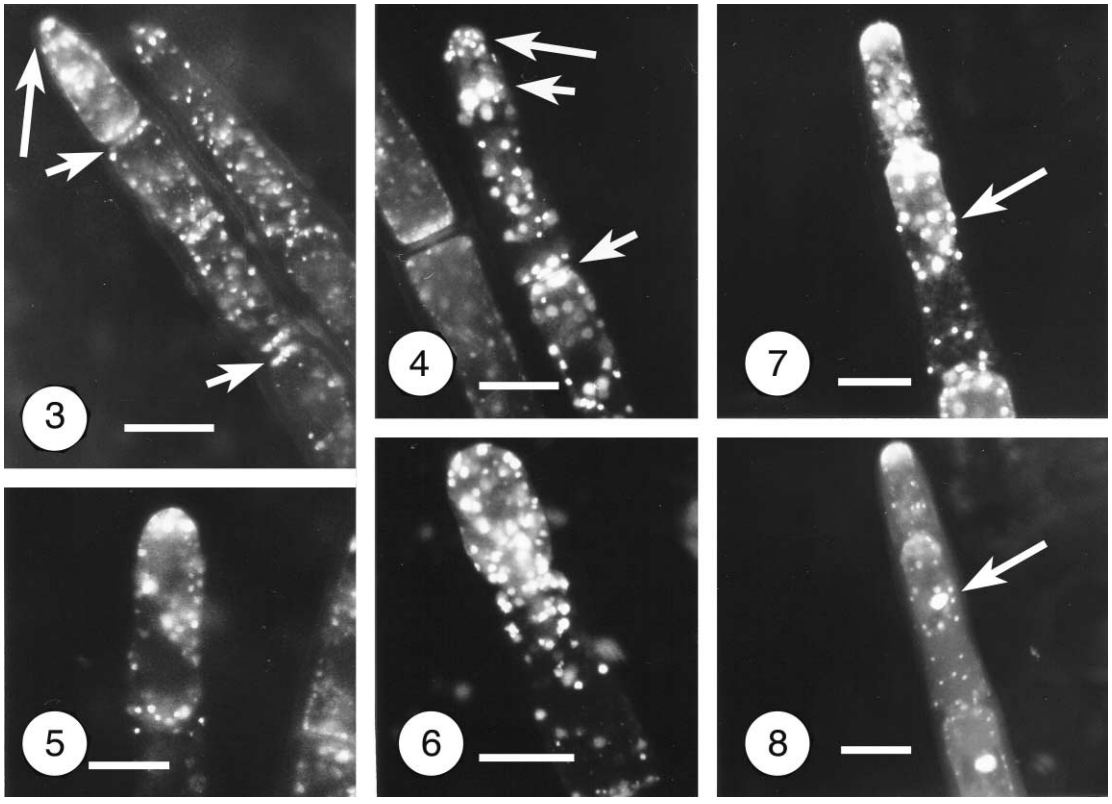
Apical cells in young filaments: Apical cells of immature filaments varied from < 5 μm to 65 μm long (Fig. 1) and had numerous mitochondria (Table 1, Figs 3-8). When a new apical cell formed (Stage I), mitochondria were evenly distributed (Fig. 3), with a slight concentration at the cell apex. Most mitochondria were small and single, and appeared as small circular dots, although a few reticulate complexes were occasionally present. In small, newly formed cells, mitochondria were difficult to quantify because of the numbers and densities of mitochondria. As apical cells doubled in size, mitochondria gradually increased, and by Stage II (Fig. 4) there was a 50% increase in single mitochondria and a three to four times increase in mitochondrial complexes (Table 1). Despite significant increase in apical cell size between Stages III to IV (from ca. 30 to 60 μm in length) (Figs 5-8), single mitochondria only slightly increased and mitochondrial complexes slightly decreased. By Stage IV, however, there was a decrease in number of mitochondrial complexes (Table 1).

During apical cell elongation mitochondria changed position and there were periods when they were concentrated in the upper, middle or lower portions. In Stages I and IV (post and pre-cytokinesis, respectively), mitochondria were concentrated in the upper portions of apical cells. During the elongation phase (Stages II-III) they were concentrated in the cell bottom or middle. Mitochondria in the upper half of the cell provided the complement for the apical cell following cytokinesis. Stage II apical cells, while comprising less than half the eventual cell length (and volume), had ca. 80% of the mitochondrial complement of fully elongated apical cells (Table 1).

Apical cells in mature filaments: Fully elongated apical cells in mature filaments were larger than in young filaments (Fig. 1). Thus, the largest apical cells prior to cytokinesis were 65 μm and 125 μm for immature and mature thalli, respectively. Consequently, apical cells from mature filaments were considered in six rather than five size categories (i.e., Stages I-VI). As with immature filaments, mitochondria in some of the smallest Stage I

Table 1. *Colaconema caespitosum*. Mitochondrial numbers in upper and lower or upper, middle and lower portions of apical cells of immature filaments at five stages of development. Note: Stage I not counted because of density of mitochondria. See Fig. 1 for ranges of cell sizes. Numbers indicate mean ± s.d.

Stage	Position	Single Mitochondria	Total single Mitochondria	Mitochondrial Complexes	Total Mitochondrial Complexes
II	1	40.7 ± 4.5		0.6 ± 0.6	
	2	20.3 ± 2.8	61.0 ± 6.8	2.8 ± 0.9	3.4 ± 1.3
III	1	31.5 ± 4.6		3.3 ± 0.8	
	2	47.5 ± 5.0	78.9 ± 7.3	3.9 ± 1.1	7.1 ± 1.3
IV	1	25.9 ± 5.1		3.1 ± 1.1	
	2	48.1 ± 5.2		6.0 ± 0.8	
	3	21.5 ± 2.8	95.3 ± 6.3	3.3 ± 0.7	12.3 ± 1.6
V	1	53.7 ± 6.1		1.0 ± 0.9	
	2	29.5 ± 5.1		3.7 ± 1.2	
	3	16.5 ± 3.6	99.8 ± 7.0	3.2 ± 2.2	7.0 ± 2.2



Figs 3-8. Mitochondrial labelling in apical and adjoining cells of young thalli of *Colaconema caespitosum*. **Fig. 3.** Small apical cell (long arrow) with dense staining at apex and subapical cells with concentrations of mitochondria along end walls (short arrows). **Fig. 4.** Stage II apical cell (long arrow) with diffuse mitochondria showing single and clusters of mitochondria. Note dense aggregations of mitochondria (short arrows) in subapical cells. **Fig. 5.** Portion of Stage V apical cell with clusters of mitochondria at apex and more scattered mitochondria elsewhere. **Fig. 6.** More diffuse arrangement of mitochondria in Stage IV apical cell. **Figs 7-8.** Apex of same filament stained for mitochondria (Fig. 7) and nuclei (Fig. 8) with many mitochondria in vicinity of nucleus (arrow). Figs 3-8, scale bar = 20 μm.

apical cells (i.e., ca. ≤ 5 μm) were not counted because of high mitochondrial densities (Figs 9, 10).

During development of apical cells there is a continuous increase in single mitochondria (ca. 400%), whereas mitochondrial complexes peaked during middle stages of development and then declined (Table 2). At each stage during apical cell development the number of mitochondria increased. Mitochondrial complexes increased until Stage IV and then declined at Stage VI (significant at $p < 0.01$, students t test). Mitochondria

Table 2. *Colaconema caespitosum*. Mitochondrial numbers in upper and lower or upper, middle and lower portions of apical cells of mature filaments at five stages of development. Note: Stage I not counted because of density of mitochondria. See Fig. 1 for ranges of cell sizes. Numbers indicate mean ± s.d.

Stage	Position	Single Mitochondria	Total single mitochondria	Mitochondrial complexes	Total mitochondrial complexes
II		33.4 ± 3.8	33.4 ± 3.8	0.5 ± 0.8	0.5 ± 0.8
III	1	29.3 ± 5.1		1.4 ± 1.2	
	2	32.8 ± 4.8	62.1 ± 7.8	0.7 ± 0.7	2.1 ± 1.6
IV	1	28.0 ± 7.0		3.9 ± 1.2	
	2	22.7 ± 3.4		2.3 ± 0.9	
	3	37.1 ± 3.6	87.9 ± 9.1	3.5 ± 1.1	9.6 ± 2.1
V	1	27.3 ± 4.3		2.9 ± 0.7	
	2	49.1 ± 4.6		2.5 ± 1.2	
	3	23.8 ± 5.6	100.2 ± 7.8	1.6 ± 0.6	7.1 ± 1.2
VI	1	62.2 ± 5.7		0.1 ± 0.3	
	2	34.0 ± 6.7		0.6 ± 0.7	
	3	24.9 ± 5.4	121.1 ± 13.6	1.1 ± 0.8	1.8 ± 1.2

were not quantified for separate regions of the cell at Stage I, since they were more or less evenly distributed. At Stage II (Fig. 10) the upper and lower positions of cells were not significantly different. Between Stages IV to VI single mitochondria were successively more numerous in the lower, middle and upper portions of the cell with 43%, 49% and 51% of mitochondria appearing in these regions, respectively (Table 2).

Mitochondrial complexes also varied within apical cells and peaked at Stages IV and V (9.6 and 7.1) and then declined by Stage VI (1.8). This decline was consistent with the low number of MC in terminal stages of apical cells of immature filaments as well as the low number of MC in newly divided apical cells. The overall correlation between numbers of single and complexed mitochondria from Stages III-VI was not significant (r = 0.062); however, in Stages IV-VI there was a strong negative relationship (r = -0.778),

Mitochondria in subapical cells of *Colaconema caespitosum*

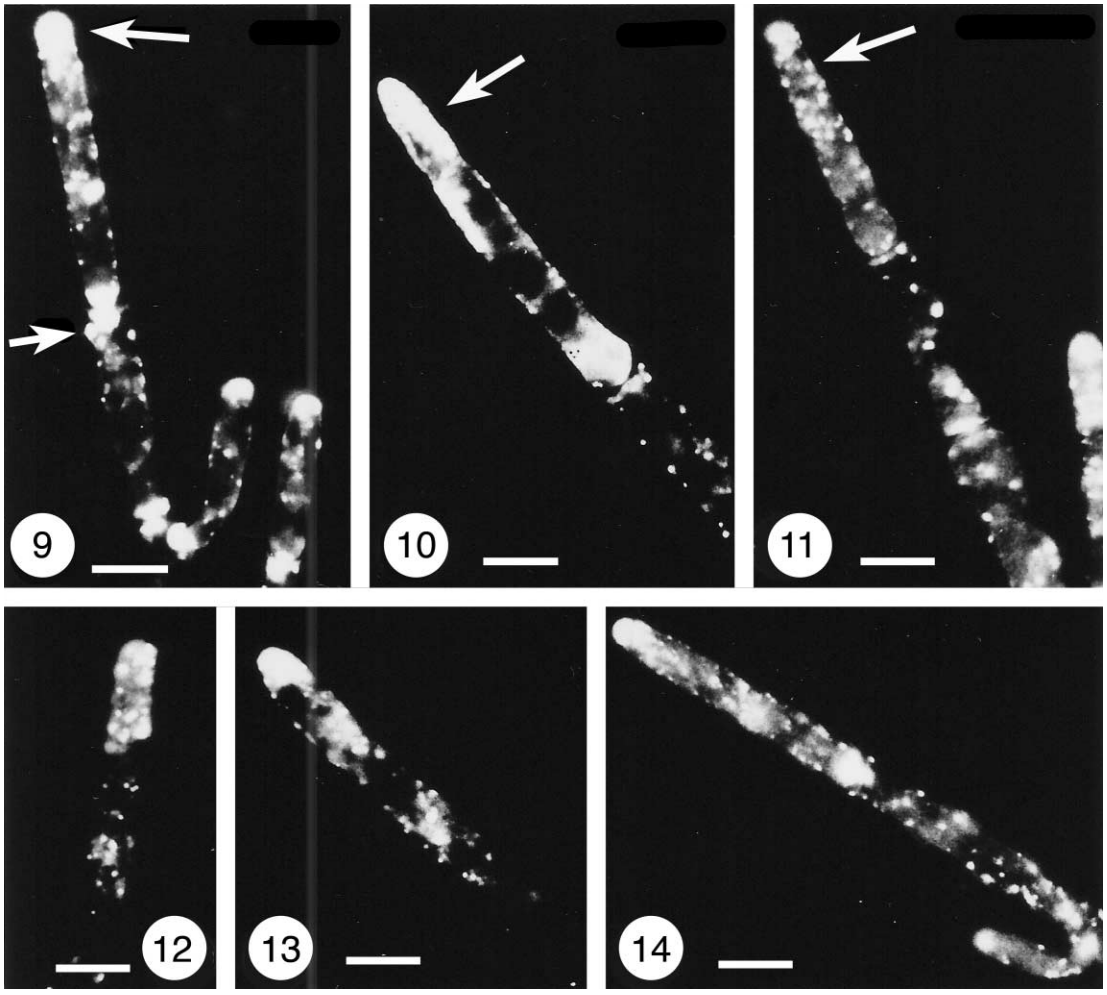
Young filaments: Single mitochondria in subapical cells were typically ovoid or spherical; in many cells they aggregated, forming large spherical complexes (Fig. 7). The number of mitochondria in subapical cells did not increase significantly during cell elongation. In most cells, mitochondria were fewer than in Stage V apical cells (Fig. 5).

Mitochondrial distribution in the first subapical cell changed with development. In newly produced cells, many mitochondria concentrated at opposite ends of the cells (Figs 6-9) with few mitochondria in the central region. They occasionally formed a dense ring particularly in the first and second subapical cells and were associated with the nucleus (Figs 8, 13, 14). In older intercalary cells, fewer mitochondria were present in the upper and central regions, and most were aggregated in a band at the cell base.

Mature filaments: The size and number of mitochondria in subapical cells were roughly similar to young filaments (Figs 9-14). They were distributed mostly at either end of subapical cells (Figs 9, 10). Mitochondria were more prominent in the upper third of subapical cells where they formed a few large reticulate structures (Figs 9, 12-14). Mitochondrial aggregations associated with lateral branch initiation were present in subapical cells (Fig. 9). These branch initials developed similarly to apical cells of primary axes.

Apical and subapical cells of *Antithamnion cruciatum*

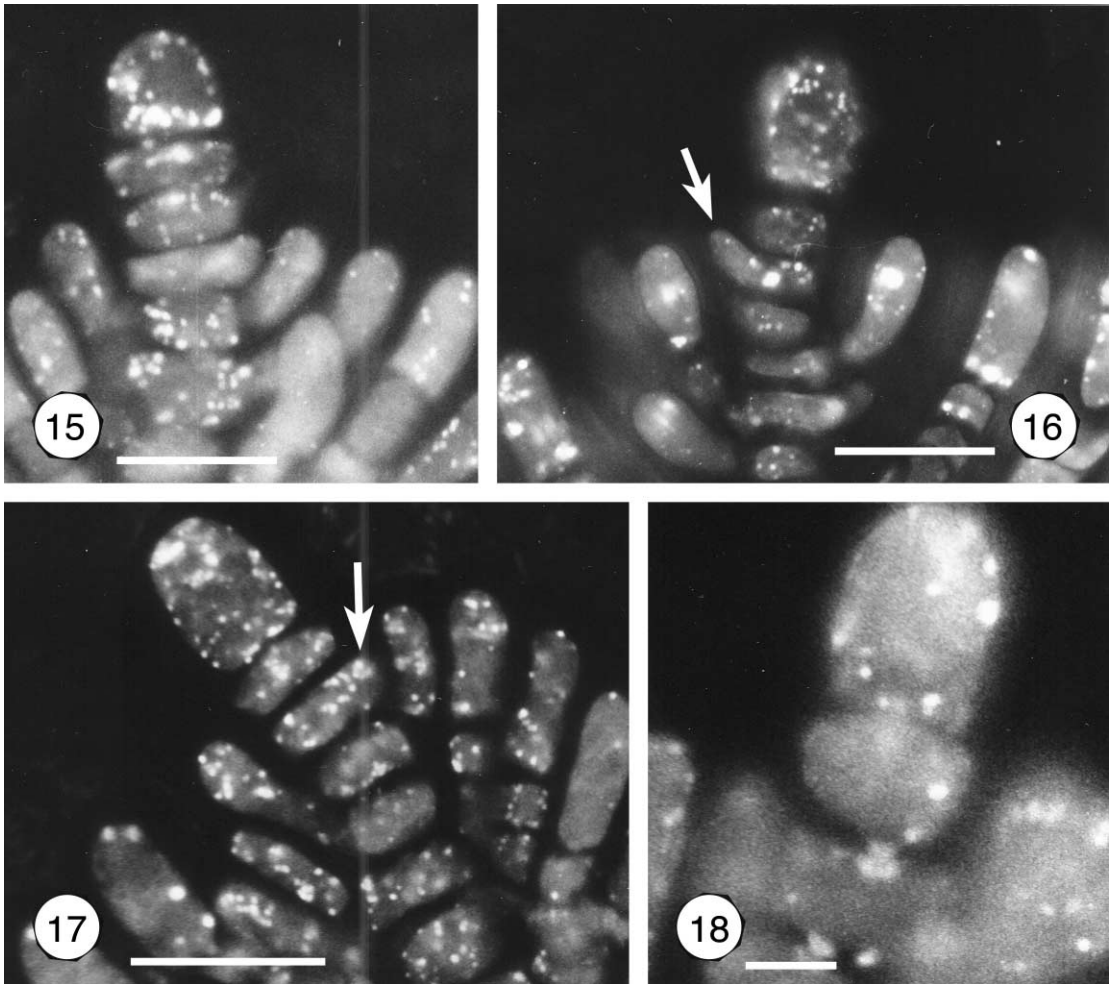
Apical cells of indeterminate (main) axes: Mitochondrial numbers increased during apical cell development from ca. 20 to 45 (Table 3). The smallest apical cells had evenly distributed mitochondria; however, during Stage I (5-7 μm) mitochondria became more concentrated at the cell base (Fig. 15). As apical cells elongated, greater numbers of mitochondria were present at cell apices (Fig. 16). This was most conspicuous in the largest apical cells where there were almost twice as many mitochondria in the upper half of the cell (Fig. 17). In these cells, mitochondria were distinctly clustered at opposite ends, while the one or two nuclei occupied the central region of the cell. Mitochondrial complexes increased during cell elongation (from ca. 2 to 5) and also



Figs 9-14. Mitochondria from apical and adjoining cells of mature thalli of *Colaconema caespitosum*. **Fig. 9.** Dense concentration of mitochondria in Stage II apical cell (large arrow), and in bud for developing branch (small arrow) with localized concentrations in subapical cells. Scale bar = 25 μm). **Fig. 10.** Stage III apical cell (arrow) with densely staining mitochondria. **Fig. 11.** Stage IV apical cell with arrow indicating midpoint of cell. Scale bar = 20 μm. **Fig. 12.** Portion of Stage V apical cell with mitochondrial aggregations in upper third of cell. Scale bar = 20 μm. **Fig. 13.** Portion of fully elongated (Stage VI) apical cell with localized aggregations of mitochondria. Scale bar = 20 μm. **Fig. 14.** Fully elongated apical cell (lateral branch is just below septum) with single and clustered mitochondria. Scale bar = 20 μm.

Table 3. *Antithamnion cruciatum*: number and distribution of mitochondria in apical cells of main axes and the first subapical cell of the main axis of tetrasporophytic thalli. Numbers indicate mean ± s.d.

Cell and stage	Position	Single Mitochondria	Total Single Mitochondria	Mitochondrial Complexes	Total Mitochondrial Complexes
Apical Cell					
Stage 1	1	8.9 ± 1.5		0.5 ± 0.5	
	2	13.7 ± 3.8	22.5 ± 4.2	1.7 ± 1.4	2.2 ± 1.4
Stage II	1	18.2 ± 2.8		2.1 ± 0.9	
	2	13.9 ± 2.8	32.1 ± 2.8	0.4 ± 0.5	2.5 ± 0.9
Stage III	1	30.1 ± 4.1		4.2 ± 0.8	
	2	16.3 ± 4.1	46.5 ± 6.7	0.5 ± 0.5	4.7 ± 0.9
Subapical Cell			21.5 ± 5.4		2.9 ± 1.1



Figs 15-18. Mitochondria in apical region of *Antithamnion cruciatum*. **Fig. 15.** Stage I apical cell with band of mitochondria along lower end of cell and with scattered, mostly single mitochondria in other cells. Scale bar = 10 μm. **Fig. 16.** Stage II apical cell with scattered mitochondria. Scale bar = 10 μm. **Fig. 17.** Stage III apical cell with many single and a few aggregations of mitochondria. Arrow indicates mitochondria in developing branch bud. Scale bar = 20 μm. **Fig. 18.** Stage III apical cell various sizes of mitochondria and numerous mitochondria out of the plane of focus in upper part of cell. Scale bar = 10 μm.

became more conspicuous at the cell tip (Table 3, Fig. 18).

All of the above data were from tetrasporophytic thalli. Similar data were also gathered from male and female gametophytic plants (Table 4). Male and female plants were remarkably similar at all stages. In addition, except for apparently lower mitochondrial numbers in the smallest apical cells (Stage I), larger apical cells (Stages II-III) were similar to tetrasporophytes. These lower counts for Stage I gametophytes are anomalous and inconsistent with post-cytokinetic numbers expected from Stage III apical cells (i.e., ca. 20 mitochondria).

Subapical cells of indeterminate axes: In general, lateral branches first appeared on the second subapical cell. Many mitochondria aggregated where the branch was to form and the balance of mitochondria was evenly distributed within cells (e.g., Fig. 17). In the first subapical cell, mitochondrial distribution had no consistent distribution pattern, with irregular concentrations of mitochondria centrally or on opposite sides of the cell. Some fused to form a spherical mass. The average number (including

Table 4. *Antithamnion cruciatum*: number and distribution of mitochondria in apical cells of male and female gametophytic thalli. See Fig. 2 for size ranges of apical cells at stages I-III. Numbers indicate mean ± s.d.

Position	Stage I	Stage II	Stage III
Female thalli (n=15)			
1		10.6 ± 2.5	30.0 ± 3.1
2		18.4 ± 3.3	10.4 ± 1.6
Total	8.7 ± 2.1	28.5 ± 4.5	40.5 ± 3.6
Male thalli (n=4)			
1		9.3 ± 1.9	28.0 ± 2.5
2		18.5 ± 2.3	10.3 ± 0.8
Total	9.8 ± 1.1	27.8 ± 1.4	38.3 ± 2.8

Table 5. *Antithamnion cruciatum*: number and distribution of mitochondria in apical cells and cells two to six in determinate axes of tetrasporophytic thalli. Numbers indicate mean ± s.d.

Position	Apical Cell			Subapical Cells				
	Cell 1			Cell 2	Cell 3	Cell 4	Cell 5	Cell 6
	Stage I	Stage II	Stage III					
1		22.3	21.0	10.5	9.3	12.1	12.1	14.9
		± 2.9	± 3.7	± 2.2	± 2.4	± 2.6	± 2.4	± 2.6
2			18.6		18.5	21.9	24.2	33.5
			± 3.1		± 3.5	± 3.6	± 3.3	± 3.6
3		22.9	21.1	13.5	11.7	17.6	16.3	19.7
		± 3.3	± 2.1	± 3.0	± 11.7	± 17.6	± 16.3	± 19.7
Total	31.3	45.2	60.7	23.9	39.5	51.5	52.5	68.1
	± 4.2	± 4.9	± 4.4	± 2.9	± 5.5	± 6.6	± 4.9	± 4.3

single and MC) was about 25 (Table 3). This variation may represent stages in branch initiation.

Apical and subapical cells of determinate branches: As with the indeterminate axes, the mitochondria in apical cells of determinate branches increased. Mitochondria were evenly distributed during apical cell growth and there was a doubling of mitochondria during cell elongation from 30 to 60 (Table 5). The mitochondrial complement of small apical cells was half that in the larger cells and half that present in the lower portion of the enlarged apical cell. As the subapical cell elongated there was a corresponding increase in mitochondria. Following cytokinesis, mitochondria were initially evenly distributed (Cell 2, Table 5); however, in cells 3-6 there was a conspicuous concentration of mitochondria in the middle third of the cell (ca. 40-50% of total mitochondria) (Table 5).

The above data was from tetrasporophytes and similar data was obtained from apical cells of male and female gametophytes (Table 6). The results were from the two sexes and the two life history phases were equivalent, even though counts for gametophytes were higher in later stages (i.e., 61 and 77 mitochondria for tetrasporophytes and gametophytes, respectively).

Table 6. *Antithamnion cruciatum*: number and distribution of mitochondria in apical cells of determinate branches of gametophytic thalli. Numbers indicate mean ± s.d.

Position	Stage I	Stage II	Stage III
Male thalli (n=15)			
1		38.7 ± 2.3	45.8 ± 3.9
2		17.9 ± 2.6	31.2 ± 3.3
Total	28.1 ± 2.7	56.5 ± 3.4	77.0 ± 5.7
Female thalli (n=3)			
1		38.5 ± 2.3	41.3 ± 2.9
2		17.0 ± 2.7	33.0 ± 1.7
Total	27.3 ± 1.8	55.5 ± 1.5	74.5 ± 5.1

DISCUSSION

Mitochondrial labeling of *C. caespitosum* and *A. cruciatum* with DASPMI and $DiOC_6(3)$ led to several generalizations. First, red algal mitochondria label similar to other algae (e.g., Chida and Ueda 1986; Hatano and Ueda 1988; Hayashi and Ueda 1989; Tornbom and Oliveira 1993), although the red algal wall seems more difficult for these fluorescent stains to penetrate than in other algae. This problem was previously noted with entry of other labeling agents (Garbary and McDonald 1998), and was overcome with the use of concentrated seawater or glucuronidase.

Both $DiOC_6(3)$ and DASPEI gave similar results when staining red algae. Although DASPEI is supposedly specific for mitochondria whereas $DiOC_6(3)$ can also stain the Golgi apparatus and endoplasmic reticulum, no differences were noted in labelling patterns of the two stains. As in previous studies (e.g., Chida and Ueda 1986), each labeled dot-like or regularly ovate structure was interpreted as a single mitochondrion. Although larger, often irregularly shaped structures were counted as single mitochondria, they were referred to as mitochondrial complexes (e.g., Chida and Ueda 1986; Hatano and Ueda 1988) and are interpreted as comprising a number of fused or aggregated organelles.

In addition to the *Rhodella maculata*, *Cyanidium caldarium*, and *Galdieria sulphuraria* (Broadwater and Scott 1986; Suzuki *et al*. 1994), where only a single mitochondrion is present, a single mitochondrion appears to characterize many unicellular algal systems (e.g., *Pyramimonas gelidicola* McFadden, Moestrup et Wetherbee, McFadden and

Wetherbee 1982; *Friedmannia israelensis* Chantanachat et Bold, Melkonian and Berns 1983; *Euglena gracilis* Klebs, Hayashi and Ueda 1989). Florideophyte red algae are not consistent with this pattern, and over 100 mitochondria may be present in apical cells alone.

Mitochondrial numbers in multicellular red algae have remained unclear since three-dimensional reconstruction of whole cells has not been undertaken. Ultrastructural studies of red algae in general suggest that numerous mitochondria are present (e.g. Tsekos *et al*. 1985; Broadwater *et al*. 1986a, b). This is consistent with the numerous mitochondria observed by Russell *et al*. (1993) and Garbary and McDonald (1998) in species of *Ceramium* and *Griffithsia*, and the 100-120 and 40-80 mitochondria shown here in apical cells of *C. caespitosum* and *A. cruciatum*, respectively. Garbary and Pei (2000b) did not count the mitochondria in monosporangia or germinating monospores of *C. caespitosum*; however, it is apparent that these developmental stages have numerous mitochondria.

We have demonstrated highly dynamic changes in mitochondrial abundance associated with the cell cycle in apical cells. In *C. caespitosum* and *A. cruciatum* there is a major increase in mitochondrial numbers. In immature filaments of *C. caespitosum* an expected doubling only partially resolved (i.e. from ca. 60-100). This might reflect the fact that the smallest apical cells were not included in the analysis because of difficulties in counting. The four times increase in mitochondria in apical cells of mature filaments is apparently not consistent with a cell cycle of doubling (cell enlargement) and then halving (during cytokinesis). However, the low number of mitochondria at the basal end of fully elongated apical cells is consistent with predicted cell cycle changes. This consistency was also observed in apical cells of *A. cruciatum* for both gametophytes and sporophytes. The only major anomaly in these data is the low number of mitochondria in the smallest size category of indeterminate apical cells in gametophytes relative to tetrasporophytes (9-10 vs. 22).

Differences in apical cell size of immature and mature filaments shown here for *C. caespitosum* were noted previously in *Acrochaetiaceae* in *Acrochaetium secundatum* (Lyngbye) Nägeli by Garbary (1979) [as *Audouinella secundata* (Lyngbye) Woelkerling. In the latter species there was a continuous increase in apical and subapical cell length from the smallest to largest plants. The similarities in mitochondrial abundance in Stage IV apical cells in both immature and mature thalli suggests a prolongation of development in mature thalli rather than a substantial change in developmental pattern. Since species of Acrochaetiaceae apparently do not become endopolyploid (Garbary and McDonald 1998), it may that the amount of nuclear DNA provides an ultimate limit on apical cell size.

All of the apical and subapical systems that we investigated in the two species have a clear correlation between cell size and mitochondrial number. Although such a relationship might be expected based on requirements of cell metabolism, *C. caespitosum* and *A. cruciatum* are the only algal systems where this has been demonstrated using quantitative data.

Differentiation of indeterminate and determinate apical cells of *A. cruciatum* is partly reflected in mitochondrial abundance in that the former have greater numbers of mitochondria (22 vs. 31 in Stage I and 46 vs. 61 in Stage III). Thus, in addition to differences in cell shape, there are differences in organelle composition between these cell types. Differentiation at the molecular-genetic level is clearly required to understand the developmental control mechanisms of these two types of apical cells.

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