

ORIGINAL ARTICLE

Fumio Takahashi · Koji Yamaguchi · Tasuku Hishinuma
Hironao Kataoka

Mitosis and mitotic wave propagation in the coenocytic alga, *Vaucheria terrestris* sensu Goetz

Received: March 31, 2003 / Accepted: June 25, 2003 / Published online: August 16, 2003

Abstract Mitosis and cytoplasmic microtubule (MT) dynamics were observed for the first time in *Vaucheria terrestris* sensu Goetz. Mitosis could occasionally be seen in part of the cylindrical coenocytic cell. The frequency of encountering cells with dividing nuclei was highest (ca 12%) 4 h after the onset of light in 12 h light/12 h dark regimes; it decreased thereafter and approached zero during the dark period. From the anterior end of every interphase nucleus a unique, long MT bundle extended. Differential-interference optics reveals that there is a filamentous structure in front of the moving nucleus. In prophase, the interphase bundle disappeared and shorter MT bundles emanated from both ends of the nucleus. In metaphase, the cytoplasmic MTs completely disappeared, probably being recycled to spindles. Continuous MTs elongated in anaphase and developed into an interzonal spindle in telophase; this elongated up to as much as 10 μm . The daughter nuclei were pushed away from each other by the interzonal spindle. Mitosis started synchronously in a relatively narrow region, and the mitotic stage propagated as a mitotic wave to adjacent regions, most frequently from tip to base. The role of the mitotic wave in tip growth and morphogenesis of a coenocytic cell is discussed.

Key words Coenocyte · Mitotic wave · Nuclear division · Tip growth · *Vaucheria*

F. Takahashi · K. Yamaguchi · H. Kataoka (✉)
Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira,
Aoba-ku, Sendai, 980-8577, Japan
Tel. +81-22-2175710; Fax +81-22-2175712
e-mail: kataoka@ige.tohoku.ac.jp

F. Takahashi
National Institute for Basic Biology, Okazaki, Japan

T. Hishinuma
Faculty of Science, Yamagata University, Yamagata, Japan

This paper is dedicated to the memory of Dr. Eiji Kamitsubo who passed away on 25 April 2003.

Introduction

In plant tissues, cell division is a prerequisite for new growth and morphogenesis. Since nuclear division is always followed by cytokinesis in multicellular plants, the significances of nuclear division and cytokinesis in growth and morphogenesis are indistinguishable. However, this is not the case in coenocytes, because mitosis is not followed by cytokinesis. Nuclei in a coenocytic cell also increase in number when the cell volume increases. There seems to be a regulation between the number and/or volume of nuclei and the volume of cytoplasm also present in coenocytes. However, little is known as to how mitosis is spatially and temporally regulated in individual coenocytes.

Although coenocytes (syncytia, plasmodia) are distributed widely among different phylogenetic groups, ranging from myxomycetes to higher plants and animals, they are divided in their organization into three groups as follows:

- Group 1: an individual is composed of a single coenocytic cell, e.g., the xanthophycean alga, *Vaucheria*; several green algae including *Boergesenia*, *Bryopsis* and *Caulerpa*; and the plasmodium of a myxomycota, *Physarum*;
- Group 2: an individual is composed of longitudinally connected cylindrical coenocytic cells, e.g., the green alga, *Cladophora*; the red alga, *Griffithsia*;
- Group 3: an individual body is composed of some coenocytes and ordinary uni-nuclear cells, e.g., internodal cells of *Chara* and *Nitella*; tapetal cells in flowers of higher plants; muscles of animals.

Of these coenocytic organisms, group 1 algae are the simplest and therefore suitable for the physiological study of coenocytes.

It was recently reported that the accumulation of nuclei in the irradiated region is indispensable to branch induction from blue-light-irradiated regions of coenocytic tubes of the xanthophycean alga, *Vaucheria* (Takahashi et al. 2001). *Vaucheria* cells form new tips by gathering nuclei into irradiated regions from adjacent non-irradiated areas,

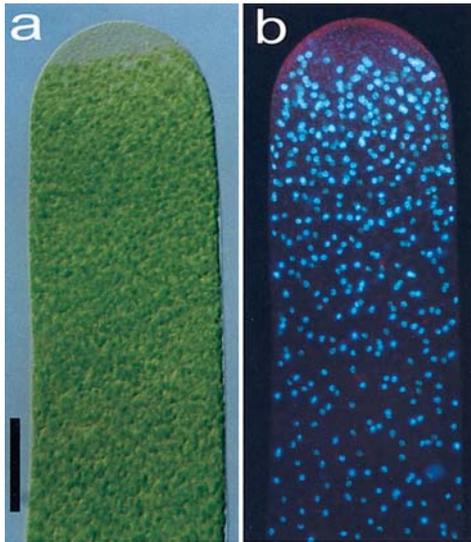
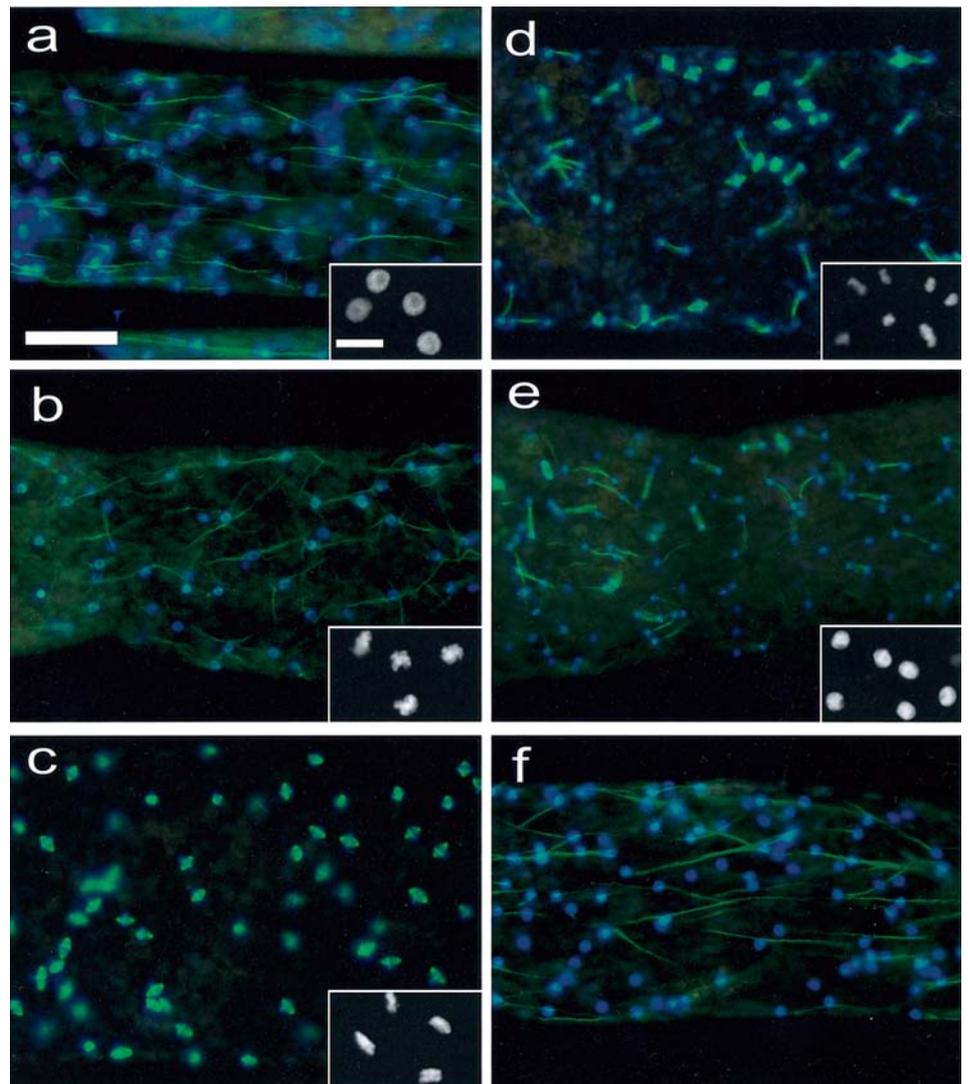


Fig. 1a,b. Massive accumulation of nuclei in the actively growing apex of *Vaucheria terrestris* sensu Goetz. **a** Differential interference contrast photomicrograph of an actively growing apex. **b** Nuclei, stained with 4', 6-diamidino-2-phenylindole, in the same region as **a**. Bar represents 50 μm

Fig. 2a-f. Changes in morphology of nuclei and microtubules (MTs) during the course of mitosis. Nuclei divide synchronously in a relatively narrow region. Representative examples of nuclei (blue) and MTs (green) at different stages of mitosis are shown. Magnified fluorescent images of nuclei at correspondent stages are shown in insets. **a** Immediately before prophase. **b** Prophase. **c** Metaphase. **d** Mixture of metaphase, anaphase and telophase. **e** Late telophase. **f** Interphase. Bar for color photographs represents 30 μm ; bar for monochromatic insets represents 5 μm



without an intervening mitosis. Dense packs of nuclei are seen not only at the presumptive site of branching, but also in the actively growing tips. These observations lead to the assumption that spatial regulation of nuclei is only short range. Similar short-range regulation by nuclei has been reported in the initiation of branching of fern protonema (Wada 1998). Such short-range control of nuclei in cytomorphogenesis may indicate that newly synthesized mRNA and proteins stay near the nuclei and do not quickly diffuse away. In *Acrosiphonia*, a green algal coenocyte belonging to group 2, a septum is formed at a subapical region of the apical cell where many nuclei gather and divide (Hudson and Waaland 1974; Aruga et al. 1996). Growth and cytomorphogenesis of coenocytes are probably either short-range controlled by nuclear accumulation or long-range regulated by mitosis occurring at an appropriate interval.

In the present article we describe the mitosis of *Vaucheria terrestris* sensu Goetz (= *V. frigida*) with immunofluorescence microscopy, and report the synchronous mitosis and mitotic waves propagating most frequently from the growing apex to the base.

Materials and methods

Plant material and culture methods

The freshwater xanthophycean alga, *Vaucheria terrestris* sensu Goetz var. *terrestris* C. A. Agardh (= *V. frigida* Roth.) was subcultured as previously described (Kataoka 1981, 1987; Takahashi et al. 2001). Thalli were transferred to fresh medium and further cultured for 4 weeks. The cells were cut into fragments of about 20 mm in length and incubated in a sterile plastic dish (60 mm × 15 mm) (Valmark, Canada) until wounds healed. Experiments were conducted with those cell fragments bearing only one actively growing tip. Unless otherwise stated, the alga was cultured in an incubator (Nippon Medical and Chemical Instruments, Osaka) at 20 ± 1 °C with 12 h light/12 h dark regimes. White light at an intensity of approximately 20 μmol m⁻² s⁻¹ was used for illumination.

Fluorescence microscopy

Fixation and immunostaining for fluorescence microscopy are essentially the same as described before (Takahashi et al. 2001). Briefly, specimens were fixed in a solution of 1.8% paraformaldehyde and 0.2% glutaraldehyde, or 1.0% glutaraldehyde, in piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES) buffer (20 mM PIPES buffer, pH 7.0, containing 0.5 mM phenylmethylsulfonyl fluoride and 5 mM ethylene glycol-*O,O'*-bis(2-aminoethyl)-*N,N,N',N'*-tetraacetic acid (EGTA), 5 mM MgSO₄ and 1.0% dimethylsulfoxide). After washing with PIPES buffer, fixed specimens were cut into about 500 μm-long segments with sharp dentist's scissors. Specimens were then treated with 2.0% Triton X-100 to decrease autofluorescence of chlorophylls. Anti-chick β-tubulin mouse antibody and secondary fluorescein isothiocyanate-conjugated (FITC-conjugated) anti-mouse immunoglobulin G (Amersham) were used to stain microtubules (MTs). 4', 6-Diamidino-2-phenylindole (DAPI) (Sigma) was used to stain nuclei either solely or after the immunostaining. For the analysis of mitotic waves, straight algal tubes of length 20–30 mm, having only one growth point, were fixed and stained only with DAPI without cutting into fragments. Other conditions for fixation were the same as above. Epifluorescence images were observed with Axioskop and Axioplan 2 microscopes (Zeiss, Germany). Cells were also observed with differential interference contrast (DIC) optics (Fig. 1, with Axioskop [Zeiss]; and Fig. 3c–e, with BF-DIC [Olympus]).

Results

Nuclei

As Fig. 1 shows, nuclei are densely accumulated in the lower half of an actively growing apical dome of *Vaucheria*. In the apical dome, cytoplasm is thickly accumulated. From the

extreme tip, however, nuclei and chloroplasts are excluded (Fig. 1a). The transparent apical cap region also contains numerous mitochondria, which are recognized as tiny fluorescent spots (Fig. 1b). The density of nuclei is low in the proximal regions or in tips that cease growth (data not shown). While it widely varies from cell to cell, the average nuclear density in the proximal region is about 1,000 nuclei per 1-mm-long *Vaucheria* tube with diameter 70 μm (Takahashi et al. 2001).

Mitosis and cytoplasmic MTs

Nuclei and MTs were double stained with DAPI and FITC-conjugated β-tubulin antibody. Interphase nuclei are most frequently observed in the specimens fixed during the light period. Figure 2 shows merged photographs of nuclei and MTs, which are arranged in order of mitotic stages. Every interphase nucleus (Fig. 2a, f) has one long MT bundle. There is almost no MT bundle without an attached nucleus at one end. As previously reported (Ott 1992; Takahashi et al. 2001), the nucleus-attaching MT bundles run parallel to the cell axis.

When nuclei start to divide, the long MT bundles become wavy and disorganized and are then destroyed (Fig. 2a, b). Several short MT bundles emanate from both ends of the prophase nucleus. However, the exact time course and dynamics of cytoplasmic MTs during transition from interphase to prophase are not yet known. The interphase nuclei shown in the inset of Fig. 2a are probably in the G₂ stage, as guessed from their size. When mitosis begins, nucleoli disappear and the profiles of chromonemata become irregular (inset of Fig. 2b).

As shown in Fig. 2c, MTs completely change into spindles and there are no cytoplasmic MTs in the region that is occupied by metaphase nuclei. Most metaphase nuclei are seen as short rods with rough contours (inset of Fig. 2c), revealing that most spindles orient parallel to the cell surface and that chromosomes are aligned at the equatorial plane of the spindle. Since no MT bundles other than the spindle can be seen in this area, cytoplasmic MTs seem to be depolymerized and recycled into spindle MTs, which are surrounded by nuclear envelopes.

Chromosomes are then separated from each other toward the spindle poles during anaphase and telophase (Fig. 2d, e). Daughter nuclei (inset of Fig. 2e) are clearly smaller than interphase nuclei shown in the inset of Fig. 2a. However, we occasionally observed interphase nuclei of about 2 μm in diameter (cf. Fig. 6c), which are thought to be in G₁ stage.

A thick MT bundle of about 10 μm in length, or the interzonal spindle, is observed between the daughter nuclei (Fig. 2d, e). The interzonal spindles are clearly shorter than the interphase MT bundles. As seen in Fig. 2e, both ends of the interzonal spindles are abruptly cut and do not reach the centrosomes. Obviously, in the gaps between an interzonal spindle and two centrosomes there are condensing daughter nuclei. As *Vaucheria* exhibits close nuclear division (e.g., Ott and Brown 1972), the nuclear envelope

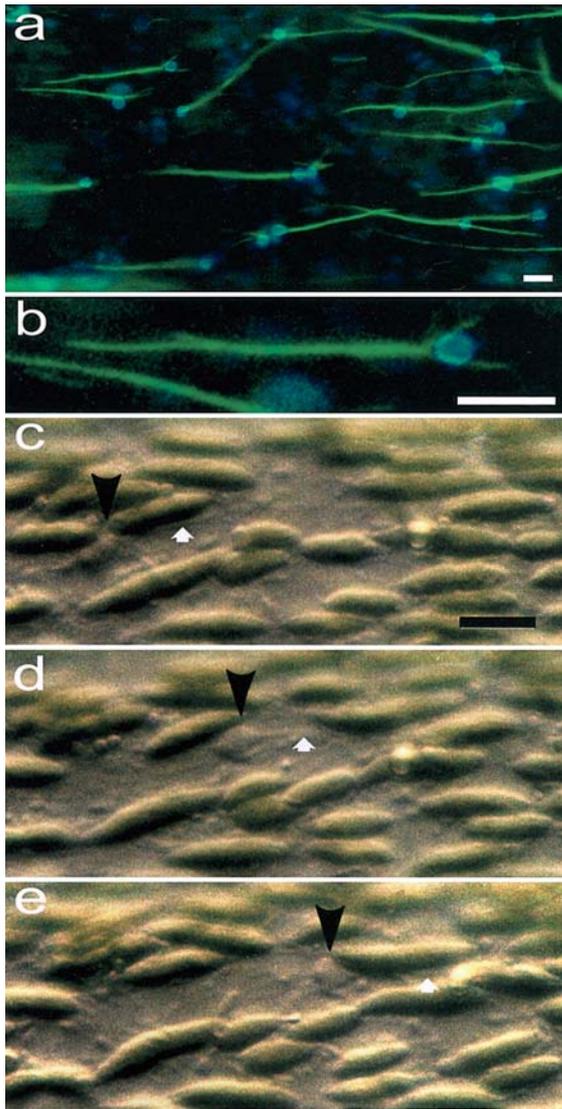


Fig. 3a–e. Interphase nuclei with long anterior MT bundles. **a** Each long MT bundle that runs parallel to the cell axis extends from a nucleus. **b** A part of **a** is magnified. Besides an anterior MT bundle, two posterior short bundles extending along the nucleus are seen. Bars in **a** and **b** represent 10 μm . **c–e** Differential interference contrast photomicrographs of a nucleus with anterior filamentous structure taken at intervals of 30 s. Black arrowheads nucleus, white arrows filamentous structure extending in front of the nucleus. Chloroplasts were previously removed from the field of view by irradiating with intense light (photoavoidance). Bar in **c** represents 10 μm

remains intact during the nuclear division. The interzonal spindle should be surrounded by nuclear envelope.

The number of chloroplasts and nuclei were reduced by keeping the alga under dim white light for several days. In this condition, the nucleus-MT bundle complexes could be much more clearly observed with fluorescence microscopy (Fig. 3a, b). The interphase nuclei often take a pyriform shape, and a long MT bundle extends from the anterior end. The average length of the anterior MT bundles shown in Fig. 3a is $41.6 \pm 5.18 \mu\text{m}$ ($n = 14$). It varies, however, from cell to cell. A strongly magnified photograph (Fig. 3b) reveals that two rear short bundles extend from the head of

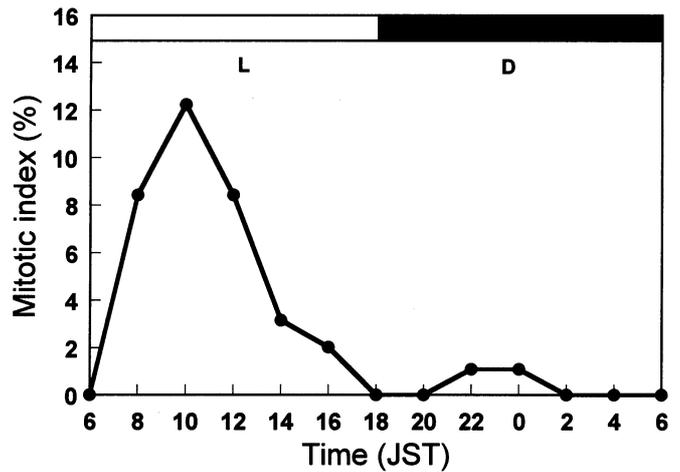


Fig. 4. Mitotic index over 1 day. More than 100 cells were inspected every 2 h during a 12 h light/12 h dark cycle and the proportions of cells in which mitosis were found are plotted versus time of day. Light period was from 0600 to 1800 hours

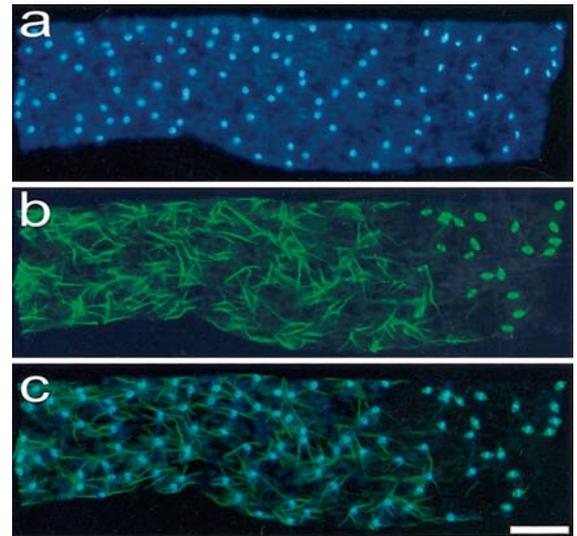


Fig. 5a–c. Prophase and metaphase are adjacent to each other. **a** Nuclei; prophase nuclei can be distinguished from interphase nuclei by their rough profiles. **b** MTs; four short MT bundles emanate from each prophase nucleus. **c** Superimposition of **a** and **b**. The long anterior MT bundles extending from interphase nuclei are not observed in prophase. Bar represents 30 μm

the nucleus, besides the anterior MT. The anterior MT bundle is found to be composed of 20–30 MTs (data not shown). The anterior MT bundle apparently corresponds to the filamentous structure observed with time-lapsed DIC optics (Fig. 3c–e).

Mitotic wave

The frequency of cells with dividing nuclei (mitotic index) was the highest 4 h after the beginning of the light-period (Fig. 4). Then it rapidly decreased and almost no mitosis was observed during the dark period.

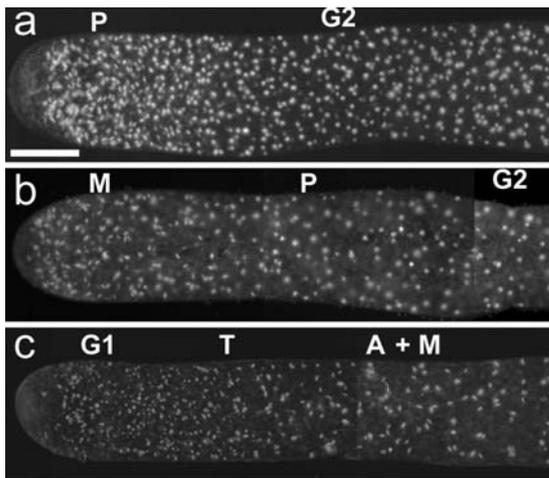


Fig. 6a–c. Basipetally propagating mitotic wave. **a** Mitosis has just started at the apex, while in the basal region nuclei are still in the interphase. **b** When nuclei in the subapical region start to divide, nuclei in the apical region enter metaphase. **c** When nuclei in the basal region are in metaphase and anaphase, nuclei in the subapical region are in telophase and, in the most apical region, nuclei have just finished their mitosis. Bar represents 50 μm

When dividing nuclei were found in *Vaucheria terrestris* sensu Goetz, the area of the nuclei in the same mitotic phase was narrow, being at the widest a few hundred micrometers. In the region next to this area, however, we saw either a zone consistent with that of the previous stage or the next mitotic stage as shown in Fig. 5. To double-stain nuclei and MTs we had to cut the cells into approximately 500 μm -long fragments after fixation with aldehydes, so that the tubulin antibody could sufficiently penetrate into the cytoplasm. This treatment, however, impeded observation of the wider area and the contiguity of mitosis within a cell. Thus, for the analysis of distribution and propagation of mitosis, we skipped the immunostaining of MTs. After culture under 12 h light/12 h dark regimes for 1 week, cell fragments 20–30 mm long that possessed a single actively growing apex were selected during a period of 4–6 h after the onset of light. The selected cells were fixed and stained with DAPI. Representative examples of the basipetally propagating mitotic wave are shown in Fig. 6. In the cell shown in Fig. 6a, the apical region (40–50 μm) is occupied by many prophase and a few metaphase nuclei, while the basal region is occupied by interphase nuclei. In Fig. 6b, the apical 60- μm region is occupied almost exclusively by metaphase nuclei, and prophase and large interphase nuclei (probably in G2) take proximal regions. The clearest example is Fig. 6c: from the very tip to the basal region, small nuclei, probably in the G1 stage, telophase, anaphase and metaphase occur in regular order. Such topological arrangement of mitotic stages can only be explained by the assumption that mitosis starts at very apex and propagates towards the base, as schematically drawn in Fig. 7.

Although basipetally propagating mitotic waves formed the great majority, apparently acropetal propagation (Fig. 8) and/or bipolar propagation (i.e., mitosis starting at an intermediate position of the cell and propagating to both

Fig. 7. Schematic drawing explaining the basipetally propagating mitotic wave. If the mitotic wave starts at a tip and propagates towards the base, mitotic stages observed in a given specimen must be arranged in the order shown here

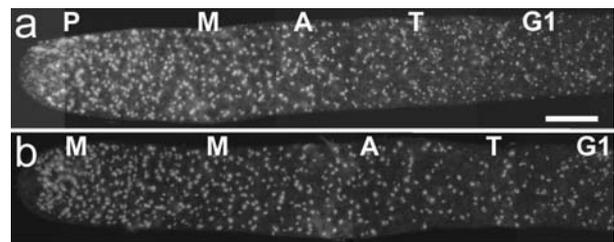
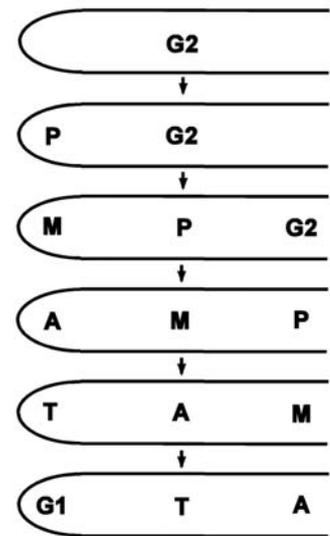


Fig. 8a,b. Examples of mitotic waves apparently propagating in the reverse direction. **a, b** Mitotic stages of nuclei in basal regions are much advanced, indicating that the mitotic wave propagates towards the apex. Cells used here were much longer than those in Fig. 6. It is not certain whether there is another growing apex at the other end, or whether these cells have growing branches in more basal regions. Bar represents 50 μm

right and left directions), were occasionally observed. This was especially the case when the cells were not previously trimmed.

Discussion

Nuclear movement and nuclear division

In *Vaucheria*, tip growth and cytomorphogenesis require a high density of nuclei, and this is achieved by transport of nuclei from proximal or adjacent region (see Fig. 1 of Takahashi et al. 2001). It is obvious, however, that the nuclear density of the cell would decrease in consequence of rapid increase in cell volume. The decrease in nuclear density would bring about a shortage of nuclei, and finally lead to the cessation of tip growth and cytomorphogenesis. In the present study we demonstrated that a semi-synchronous nuclear division often took place in the coenocytic tube of *Vaucheria*. *Vaucheria* probably maintains the nuclear density within a cell by regulating the frequency of semi-synchronous nuclear division, so as to keep the ability

to continue tip growth and morphogenesis. In this sense, both nuclear movement and nuclear division cooperate in tip growth and cytomorphogenesis.

Mitosis of *Vaucheria*

The nuclei of *Vaucheria terrestris* sensu Goetz are similar in size to those of *V. litorea* (Ott and Brown 1972), but they are considerably smaller than those of *Arabidopsis* (Chytilova et al. 2000) or *Adiantum* protonema (Wada 1998). Nevertheless, since coenocytes have numerous nuclei within a cytoplasmic continuum, the comparison of the size of individual nuclei with those of uni-nuclear cells may be meaningless. As to the distribution of nuclei, there is an interesting analogy between the shoot apex of higher plants and tip-growing *Vaucheria* cells. Similarly to the meristem of a shoot apex, numerous nuclei accumulate in the apical dome of actively growing *Vaucheria*. Although the dense accumulation of nuclei in the apical region of *Vaucheria* would mainly be achieved by transportation of nuclei from proximal parts of the cell, it must be partially due to mitosis occurring in the apical region (Fig. 7). The maximum frequency of encountering cells with dividing nuclei was 12% (Fig. 4). This value looks very low at a glance, but almost corresponds to mitotic indices reported in meristems of dicots (Couot-Gastelier 1978; Laufs et al. 1998).

Characterization of mitosis and nucleus-MT relationship

Combining our present results with electron-microscope observations of Ott (1992), the morphology of nuclei and dynamics of MTs during the course of mitosis are summarized below.

In interphase, the long interphase MT bundles extend from the anterior end of each nucleus (Figs. 2a, f and 3a, b). We have observed that there is a fibrous structure in front of the moving nucleus (Fig. 3c–e). Ott (1992) described many longitudinally running MT bundles without associated nuclei in *Vaucheria longicaulis*. We found in *V. terrestris* sensu Goetz, however, that each and all of the interphase MT bundles connect to one nucleus at one end. From our observations, we hypothesize that the nucleus-MT bundle forms a complex and this complex glides on a substratum with the bundle ahead. The interphase MTs disappear when the nuclei enter prophase. If the interphase MT bundles are essential as a motile apparatus, nuclei cannot migrate during mitosis. The following finding may support the immovability of nuclei during mitosis, namely that when apical nuclei enter mitosis, the dividing nuclear mass is left behind the advancing, continuously growing apex (see Fig. 6c). Figure 5 is also indicative of nuclear immovability during mitosis, because if dividing nuclei are translocated, the boundary between prophase and metaphase becomes unclear.

In interphase, two short, and thin rear MT bundles elongate from the anterior end of the nucleus (Fig. 3b).

In prophase, the anterior MT bundles disappear and characteristic, short MT bundles, approx four in number,

emanate from both poles of the spindle (Figs. 2b and 5). We consider that these four MT bundles originate from the rear MT bundle observed in interphase (Fig. 3). Upon duplication of the centriole pair, the bundles would have also duplicated and translocated to the rear end of the prophase nucleus together with the new centriole pair. Since no similar structures has been found previously in other plants or animals, further detailed studies are urgently needed.

In metaphase, the mitotic spindle is centric and closed, as in many other coenocytic green algae, such as *Boergesenia* (Itagaki and Ogawa 1994) and *Valonia* (Staves and La Claire II 1985). Chromosomes are arrayed in an equatorial plane of the spindle (Figs. 2c and 5). Besides spindles, no MTs are seen in the cytoplasm. This is in contrast to *Vaucheria litorea* (Ott and Brown 1972) where the long anterior MT bundles remain until metaphase and collapse during anaphase. This is clearly not the case in *V. terrestris* sensu Goetz. As *Vaucheria* exhibits closed-type mitosis, spindles must be surrounded by nuclear envelopes. This means that a metaphase nucleus should have to take up tubulin molecules through nuclear pores of the nuclear envelope.

In anaphase to telophase, two sets of chromosomes, observed as two short parallel rods (inset of Fig. 2d), move towards the spindle poles. A thick MT bundle called the “interzonal spindle”, elongating up to 10 μm , is seen between the two daughter nuclei. The interzonal spindle is thought to originate from continuous MTs that connect the two poles during metaphase and anaphase. Ott and Brown (1972) described the cutting of continuous MTs in telophase at the boundaries of daughter nuclei by invaginating inner nuclear envelopes. Our observation (Fig. 2e) is consistent with this description, i.e., the interzonal spindles do not reach the daughter nuclei.

Some of the interzonal spindles are broken at the midpoint (Fig. 2e). This may suggest that the interzonal spindle becomes two interphase MT bundles, although an alternative possibility, that the interphase MT bundles are newly built up from the tubulin pool, cannot be excluded. To distinguish between these, detailed electron-microscope studies on localization and migration of centrioles during the course of mitosis will be required.

Mitotic wave

Various propagation patterns of mitosis have been reported in phylogenetically different algal coenocytes, namely, mitoses occurring synchronously, semi-synchronously in limited regions, and non-synchronously, independently of adjacent nuclei. Synchronous mitoses are found in a red alga, *Griffithsia* (Goff and Coleman 1987), and in a green alga, *Hydrodictyon* (Marchant and Pickett-Heaps 1970). In *Griffithsia*, all the nuclei at the apical region synchronously divide and the mitoses propagate as a wave towards the basal region (Goff and Coleman 1987). Recently, Brown et al. (2002) demonstrated a beautiful mitotic wave in the coenocytic (syncytial) female gametophyte of maidenhair (*Ginkgo biloba*). The mitotic wave propagates from the

oldest area (nearest to the chalaza) to the other (opposing) side. Like *Boergesenia*, nuclei of the *Ginkgo* gametophyte are fixed in the cortical cytoplasmic sheet, keeping a constant distance from each other, and therefore, cytokinesis, mitosis and interphase are sharply differentiated in the syncytium (Brown et al. 2002).

The green algal coenocytes, *Valonia* (Staves and La Claire II 1985) and *Boergesenia* (Itagaki and Ogawa 1994; Motomura 1996) exhibit semi-synchronous mitosis. In *Boergesenia*, mitosis occurs in several discrete patches (Motomura 1996). Although the patches gradually extend, it was not certain whether they expand until the whole surface area of the cell is covered. Furthermore, very different stages of mitosis coexist in a patch. By contrast, in some green algal coenocytes, *Bryopsis* (McNaughton and Goff 1990) and *Cladophora* (McDonald and Pickett-Heaps 1976), as well as in *Vaucheria litorea* (Ott and Brown 1972), nuclei divide quite independently.

We found in *Vaucheria terrestris* sensu Goetz that the nuclear division synchronizing in a limited region propagates as the mitotic wave (Figs. 6 and 8). The mitosis probably starts most frequently at a growing apex and propagates basipetally.

When longer cell fragments were used, we often observed mitotic waves propagating towards the observed tip (Fig. 8). Taking into consideration, however, that there may be other actively growing tip(s) at the other end(s) of the cell, the observed acropetal mitotic wave could be an illusion. That is, it might have started at another end, basipetally propagated and reached the end under observation. Although nothing is known to date about the mechanism of propagation of the mitotic wave, an involvement of specific diffusing transmitters, excreted from the dividing nuclei, such as CDC 2 (Nurse 1990) and cyclins (Hunt 1991), is strongly suggested in the induction and propagation of the mitotic wave.

As demonstrated in Fig. 1, in the actively growing tip there is always dense packing of nuclei and cytoplasm. It seems to be natural to expect that apical nuclei are taking leadership in triggering mitosis among all the nuclei involved in the coenocytic continuum. Mitosis most frequently occurs 4 h after the onset of light in the 12 h light/12 h dark photoperiods. Kataoka (1975a, b) reported that tip growth of *Vaucheria geminata* exhibited daily oscillation in the 12 h light/12 h dark regimes. Tip growth started, or accelerated, 1–2 h after the beginning of the dark period. Active tip growth lasted for about 14 h, but the growth rate then decreased and the alga even ceased growth by the end of the light period. Similar growth oscillation has been observed in *V. terrestris* sensu Goetz (data not shown). Taking into consideration this and that mitosis occurs during daytime, we conclude that S-phase occurs during the dark period.

Acknowledgments This study was partly supported by a Grant-in Aid for Scientific Research (number 08304045) from the Ministry of Education, Science, Sports and Culture, Japan and a grant from Takeda

Science Foundation (to HK,1997). We are grateful to the late Dr. E. Kamitsubo for the use of his personal Axioskop. We wish to thank Dr. Steen Christensen (NIBB) for helpful advice.

References

- Aruga H, Motomura T, Ichimura T (1996) Immunofluorescence study of mitosis and cytokinesis in *Acrosiphonia duriuscula* (Acrosiphoniales, Chlorophyta). *Phycol Res* 44:203–213
- Brown RC, Lommon B, Nguyen H (2002) The microtubule cycle during successive mitotic waves in the syncytial female gametophyte of ginkgo. *J Plant Res* 115:491–494
- Chytilova E, Macas J, Sliwinka E, Rafelski SM, Lambert GM, Galbraith DW (2000) Nuclear dynamics in *Arabidopsis thaliana*. *Mol Biol Cell* 11:2733–2741
- Couot-Gastelier J (1978) Etude quelques modalités de la croissance des bourgeons axillaires de la fève *Vicia faba* L. libérés de la contrainte apicale. *Z Pflanzenphysiol* 89:189–206
- Goff LJ, Coleman AW (1987) The solution to the cytological paradox of isomorphy. *J Cell Biol* 104:739–748
- Hudson PR, Waaland JR (1974) Ultrastructure of mitosis and cytokinesis in the multinucleate green alga *Acrosiphonia*. *J Cell Biol* 62:279–294
- Hunt T (1991) Cyclins and their partners: from a simple idea to complicated reality. *Semin Cell Biol* 2:213–222
- Itagaki T, Ogawa S (1994) Mitosis in coenocytic green alga *Boergesenia forbesii* (Harvey) Feldmann (Siphonocladales, Ulvophyceae). *J Plant Res* 107:9–15
- Kataoka H (1975a) Phototropism of *Vaucheria geminata*. I. The action spectrum. *Plant Cell Physiol* 16:427–437
- Kataoka H (1975b) Phototropism of *Vaucheria geminata*. II. The mechanism of bending and branching. *Plant Cell Physiol* 16:439–448
- Kataoka H (1981) Expansion of *Vaucheria* cell apex caused by blue or red light. *Plant Cell Physiol* 22:583–595
- Kataoka H (1987) The light-growth response of *Vaucheria*. A condition *sine qua non* of the phototropic response? *Plant Cell Physiol* 28:61–71
- Laufs P, Grandjean O, Jonal C, Kieu K, Traas J (1998) Cellular parameters of the shoot apical meristem in *Arabidopsis*. *Plant Cell* 10:1375–1389
- Marchant HJ, Pickett-Heaps JD (1970) Ultrastructure and differentiation of *Hydrodictyon reticulatum*. I. Mitosis in the coenobium. *Aust J Biol Sci* 23:1173–1186
- McDonald KL, Pickett-Heaps JD (1976) Ultrastructure and differentiation in *Cladophora glomerata*. I. Cell division. *Am J Bot* 63:592–601
- McNaughton EE, Goff LJ (1990) The role of microtubules in establishing nuclear spatial patterns in multinucleate green algae. *Protoplasma* 157:19–37
- Motomura T (1996) Cell cycle analysis in a multinucleate green alga, *Boergesenia forbesii* (Siphonocladales, chlorophyta). *Phycol Res* 44:11–17
- Nurse P (1990) Universal control mechanism regulating onset of M-phase. *Nature* 344:503–508
- Ott DW (1992) The role of the cytoskeleton in organelle translocation in *Vaucheria longicaulis*. In: Menzel D (ed) *The cytoskeleton of the algae*. CRC, Boca Raton, pp 256–272
- Ott DW, Brown RM Jr (1972) Light- and electron microscopical observations on mitosis in *Vaucheria litorea* Hofman ex C. Agardth. *Br Phycol J* 7:361–374
- Staves MP, La Claire II JW (1985) Nuclear synchrony in *Valonia macrophysa* (Chlorophyta): light microscopy and flow cytometry. *J Phycol* 21:68–71
- Takahashi F, Hishinuma T, Kataoka H (2001) Blue light-induced branching in *Vaucheria*. Requirement of nuclear accumulation in the irradiated region. *Plant Cell Physiol* 42:274–285
- Wada M (1998) Branch formation induced by microbeam irradiation of *Adiantum protonemata*. *J Plant Res* 111:587–590