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Colchicine-Induced Expansion of *Vaucheria* Cell Apex. Alteration from Isotropic to Transversally Anisotropic Growth

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The tip-growth of *Vaucheria geminata* was analyzed. The elemental rate of surface expansion (RERE) at the very apex of this alga cell reaches ca. 100% min^{-1}. The expansion is almost isotropic; i.e. the both meridional and latitudinal components of RERE are almost equal. An antimicrotubular reagent, colchicine, caused expansion at the actively growing cell apex of this alga. This drug did not change the surface expansion rate, but altered the polarity of cell wall expansion from isotropic to transversally anisotropic. The orientation of cell wall microfibrils is random at the apex but axial at the basal cylindrical part of the cell. Colchicine did not change the fluence-response relationship for the first positive phototropism.

Key words: Apical expansion (Alga) — Cell growth — Colchicine — Phototropism — Polarity of cell wall expansion — *Vaucheria*.

A xanthophycean alga, *Vaucheria geminata* is composed of coenocytic tip-growing tubes (Kataoka, 1975a, 1980). The growth rate is about 200 μm hr^{-1}, and the diameter of the cylindircal cell ranges from 50 to 70 μm, which remains fairly constant under constant conditions. The phototropic bending (Kataoka, 1975a, b, 1977a, b, 1979), branch induction by blue light (Kataoka, 1975b), and the blue light-induced apical expansion (Kataoka, 1981) of this alga have been analyzed. However, a precise analysis of growth kinetics of this alga's apex has not heretofore been reported. An aim of the present paper is to describe physically the cell growth of this alga. The relative elemental rate of surface expansion (RERE) of the hemispherical apical dome was determined using the method developed by Green and King (1966).

I found that colchicine, colcemid, and vinblastine expanded the apex of this alga cell. Miller and Stephani (1971) demonstrated that spores of a fern *Onoclea sensibilis* germinated and grew to spheres in the presence of colchicine under white light, while they grew as thick protonemes if they were treated by the drug under red light or far red light. However, no kinetic analysis was made. I analyzed kinetically the apical expansion induced by colchicine by comparing the RERE of the expanded cell to that of the control cell. Colchicine seemed to bias the growth activity greatly towards the

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Abbreviation: RERE, relative elemental rate of surface expansion.

upper half of the dome. Moreover, the increased growth activity was much more obvious in the latitudinal than in the meridional direction. The present article deals with the alteration of polarity in cell surface extension at the growing apex.

Materials and methods

Biological material and culture conditions

The alga, *Vaucheria geminata* (Vauch.) De Candolle was used as the experimental material as in previous reports (kataoka, 1975a, b). The alga was precultured in a 200 m*l* Erlenmeyer flask containing 100 m*l* of culture solution (Kataoka, 1980) at 20 C under white fluorescent lamps (ca. 6 W m^{-2}). A small portion of thalli was transferred into a 6 cm petri dish 2 to 3 days prior to the experiments.

Colchicine treatment

Colchicine was supplied from E. Merck. Colcemid and Vinblastine sulfate were obtained from Calbiochem. and Sigma, respectively. All drugs were dissolved in distilled water at 10^{-2} M, then diluted to desired concentrations with the culture solution at the time of their use. The stock solutions were kept in a refrigerator in the dark, for not longer than one week. The culture solution was replaced by the drug-containing one 2 to 3 days prior to the experiment, and renewed daily, unless otherwise stated.

The relative elemental rate of surface expansion (RERE)

For determination of RERE at the tip-growing apex of the alga cell, tiny fragments of anion exchanging resin (Dowex A–1, ground in a glass homogenizer, OH^--type, Kataoka, 1975a) were used as surface markers. A drop of the resin suspension was gently added to the petri dish in which the thalli were laid. Inasmuch as the apex is extremely sensitive to mechanical shock and its growth easily stopped, placement of resin particles with the aid of a micromanipulator was not applicable (cf. Green, 1965).

Movements of resin particles were analyzed from enlarged photographic prints which had been taken at intervals of 2 min. Usually, about 20 min was the time required for a marker particle close to the very apex to move down and reach the bottom of the dome. The apical dome was approximated as a hemisphere, although the real apex was more or less tapered. Thus, the diameter of the inscribing circle was ca. 10% smaller than diameter of the proximal part of the cell. Fig. 1 shows the geometry of the apex. The downward velocity of a marker relative to the apex increases with increasing distance along the meridian from the very apex and reaches almost a maximum at the base of the dome ($\pi R/2$). The absolute value of this maximum velocity is *per se* the alga's growth rate. The meridional component of the RERE (*m-RERE*) was determined from the movements of marker particles using the method described by Green and King (1966):

$$m\text{-}RERE = \frac{1}{\Delta m} \cdot \frac{d(\Delta m)}{dt} = \frac{1}{m} \cdot \Delta\left(\frac{dm}{dt}\right) \qquad (1)$$

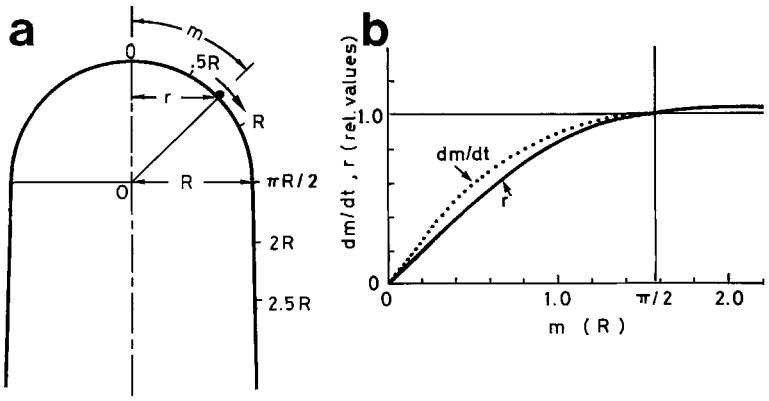

Fig. 1. Schematic diagrams showing the movement of a marker particle along *Vaucheria* cell apex. a: Geometry of an apex. Meridional distance (m) is graduated in radians (R). b: Relation of meridional velocity (dm/dt) and the radial distance (r) of a marker to meridional distance (m). Ordinate: dm/dt, and r, expressed as relative values to those at $m=\pi R/2$. For further detail, see text.

where m is the length of the meridional arc from the very apex to a point on the circle; Δm is an infinitesimally small distance along the meridian. The downward velocity of a marker (dm/dt) was expressed as values relative to the maximum, i.e. the alga's growth rate. The m-RERE was determined by drawing a curve of dm/dt against m, then by calculating the slope of the curve at every point on this curve.

Similarly, the latitudinal component of the RERE (*l-RERE*) is expressed as:

$$l\text{-}RERE = \frac{1}{\Delta l}\frac{d(\Delta l)}{dt} \tag{2}$$

where l is the latitudinal distance at the point of a marker; Δl is the infinitesimally small distance along a latitudinal direction on the surface of the dome. Since the apex is approximated as a hemispere, l equals the circumference of a circle whose radius is r (see Fig. 1a). Thus, the equation (2) can be simplified as:

$$l\text{-}RERE = \frac{1}{r}\cdot\frac{dr}{dt} \tag{3}$$

and

$$l\text{-}RERE = \frac{1}{r}\cdot\frac{dr}{dm}\cdot\frac{dm}{dt} \tag{3'}$$

The *l-RERE* at all meridional positions were calculated from the equation (3') Green and King, 1966). Pairs of RERES were drawn against m; the distance below the bottom of the hemisphere ($\pi R/2$) was also graduated by the same scale (R). The RERE at a given position is a sum of *m-RERE* and *l-RERE*.

Other methods

Phototropic bending of colchicine-treated cells were analyzed using the same

optics and methods previously reported (Kataoka, 1975a, 1980). For observation of cell wall texture, cells were treated with 4 N NaOH for 18 hr, then neutralized with 1 M acetic acid and deproteinized with 1% Na-hypochlorite solution for 10 hr. The cell wall specimens were observed with a Nomarski differential interference microscope (Zeiss) and a polarized light microscope (Nikon).

Results

Colchicine-induced expansion at the apex

Colchicine caused a marked expansion at the apex of the *Vaucheria* cell. However, the effect was limited to the actively growing apices; the diameter of the non-growing basal portion remained constant throughout the treatment, as was the case in blue light-induced apical expansion (Kataoka, 1981). At concentrations higher than 1 mM, the apex became spherical in shape and the growth was inevitably arrested. Below that concentration, however, the diameter gradually increased and reached a maximum of ca. 150% of the original value about 2 hr after the application of the drug. Fig. 2 shows the plumping process. When the drug was washed out, the diameter of the apex (measured at the base of the apical dome) gradually reduced to the original value (Fig. 3). Fig. 3 clearly shows that the longitudinal growth rate was reduced to about 3/4 by the application of colchicine (2×10^{-4} M). Meanwhile, the diameter increased to about 4/3. Obviously, the rate of increase in surface area remained constant during the treatment, while the rate of increase in cell volume increased to ca. 120% of the original rate. Therefore, it can be concluded here that colchicine does not change the

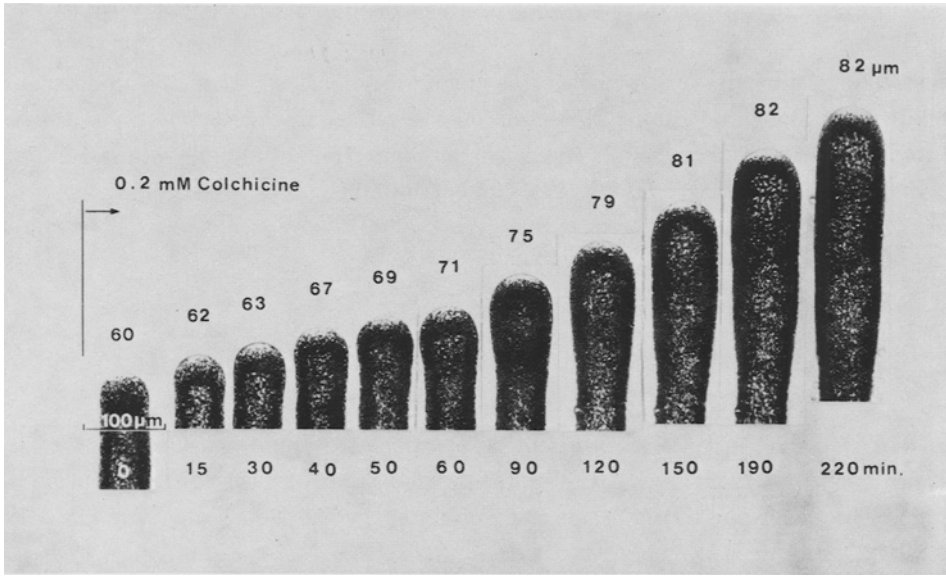

Fig. 2. Time-course of apical expansion caused by colchicine. Colchicine (2×10^{-4}M) was added at time 0. Photographs were taken with green light. Numerals above each picture is cell diameter at the base of the dome.

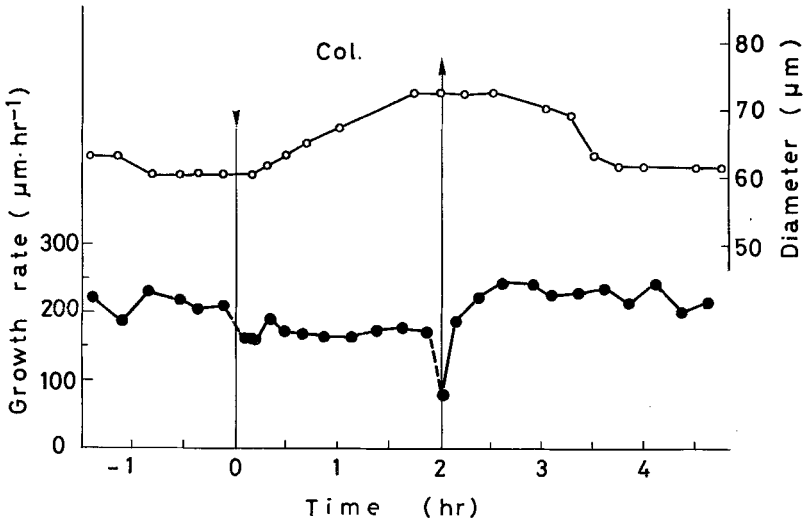

Fig. 3. Time-course of colchicine-induced expansion and change in growth rate. Period of colchicine treatment was 2 hr (between two arrows). Diameter was measured at the base of the dome.

Table 1. Diameter of the cell apex treated by colchicine and some other related drugs

Drug	Concn.	Number of cells measured	Period of treatment	Cell diameter (%)
Colchicine	2×10^{-4}M	20	3 days	171±7
	0	20	3	109±5
	1×10^{-4}M	10	2.5	134±4
	0	10	2.5	100±1
	2.2×10^{-3}M	6[1]	2	137±4
	2.2×10^{-4}M	10	2	137±8
	2.2×10^{-5}M	10	2	119±5
	2.2×10^{-6}M	10	2	114±4
	0	10	2	101±3
Colchemid	1×10^{-4}M	—[1]	2	—
	1×10^{-5}M	20	2	129±7
	1×10^{-6}M	20	2	113±4
	1×10^{-7}M	20	2	117±11
	1×10^{-8}M	20	2	106±3
	0	20	2	103±3
Vinblastine sulfate	1×10^{-5}M	—[1]	2	—[1]
	1×10^{-6}M	20	2	114±5
	1×10^{-7}M	20	2	135±9
	1×10^{-8}M	20	2	113±3
	0	20	2	100±1

[1] Growth was inhibited at that concentration.

total rate of cell wall synthesis, but possibly changes the polarity of excretion and/or expansion of wall at the apex.

Table 1 demonstrates that the degree of expansion depends on the concentration of colchicine, and that besides colchicine, colcemid and vinblastine are also effective for the expansion. The time-course of apical expansion and the morphology of the expanded apices treated by vinblastine were identical to those poisoned by colchicine or colcemid (data not shown). Although the action of vinblastine is known to be different from the latter two (Bryan, 1971), the above fact may suggest that the apical expansion is due solely to disorder or degradation of microtubule, irrespective of the mode of drug's action. Hereafter, further analyses of expansion will be limited to colchicine-treated apices.

The RERE

Fig. 4a and b are examples of respective series of photomicrographs demonstrating the movements of resin particles in the absence (a) and presence (b) of colchicine, from which REREs were calculated. Fig. 4 clearly demonstrates that the movements of markers almost cease after their arrival at the bottom of the dome. The shape of the

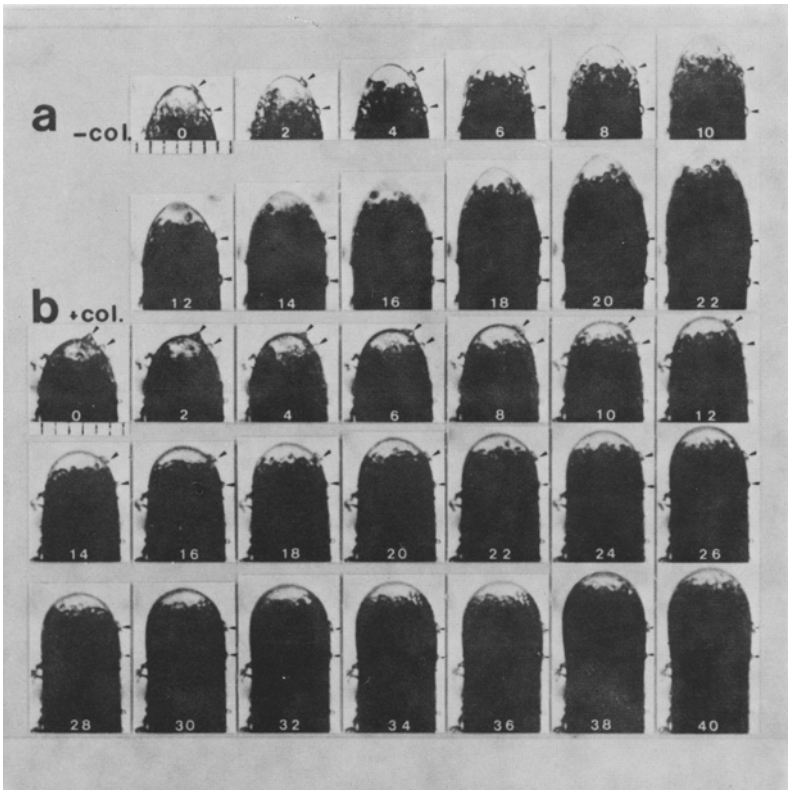

Fig. 4. Movements of resin particles along normal and colchicine-treated apices. Photographs were taken at intervals of 2 min (white numerals at the bottom of each picture). Scale of micrometer (10 μm) is superimposed on each 0 min-picture.

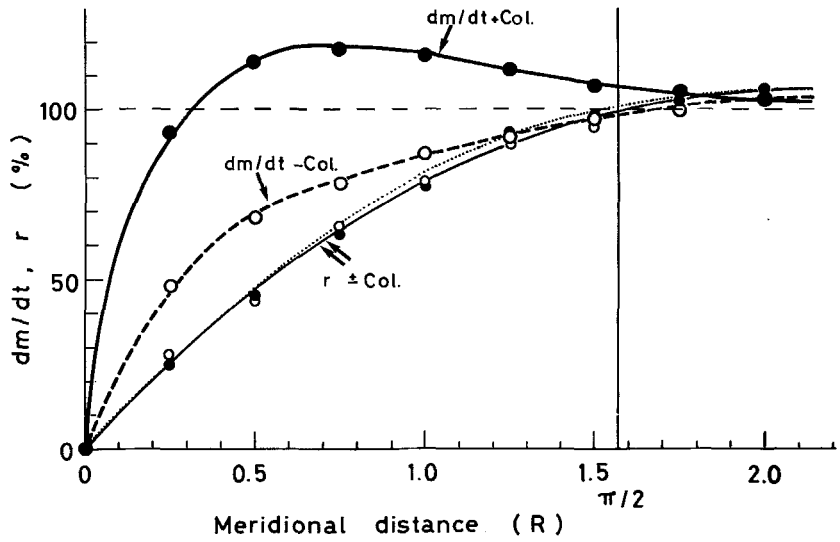

Fig. 5. Colchicine-induced shift in growth activity towards the extreme apex. Downward meridional velocity (*dm/dt*) is higher on colchicine-treated apex than on control one. Abscissa and ordinate: similar to Fig. 1b.

dome in Fig. 4a is somewhat tapered compared with that of b; rapidly growing apices are more or less cone-shaped. Most apices analyzed here were, however, much closer to an accurate hemisphere than is the case in this example.

In Fig. 5, the relative meridional velocities (*dm/dt*) and the radial distance from the axis (*r*) in the presence and absence of colchicine were drawn against the meridional distance from the very apex (*m*). The radial distance (*r*) increases following a sine-function; the two *r*-curves are overlapped by each other, indicating that colchicine does not cause any geometrical change at the apex beyond increasing the diameter. It is obvious from the figure that the *dm/dt*-values of the colchicine-treated apex are very much larger than those of the control over the whole meridian. The slope of *dm/dt* curve of colchicine-treated apex is steep until the marker arrives at 0.25 R, but becomes gentle and shows a slightly negative value below 0.8 R. Fig. 5 also indicates that in the presence of colchicine the surface expansion is almost completed within the arctic area (distal 45% region). The *m-RERE* and *l-RERE* were calculated from Fig. 5 and are shown in Fig. 6. As is clearly shown in Fig. 6a, the cell wall expansion of the normal apex is almost isotropic; although *l-RERE* are slightly larger than *m-RERE* over the whole meridian, differences are not significant. The negative values of *m-RERE* shown in Fig. 6a may partly be due to experimental error; i.e. the apex was approximated as an exact hemisphere, and the growth rate was estimated to attain the maximum at the bottom of the dome. In the real apices, increase of the diameter takes place beyond $\pi R/2$, extending as far as 2.5 R.

On the contrary, the colchicine-treated apex exhibits distinguished transversally anisotropic expansion. The negative *m-RERE* values of the colchicine-treated apex

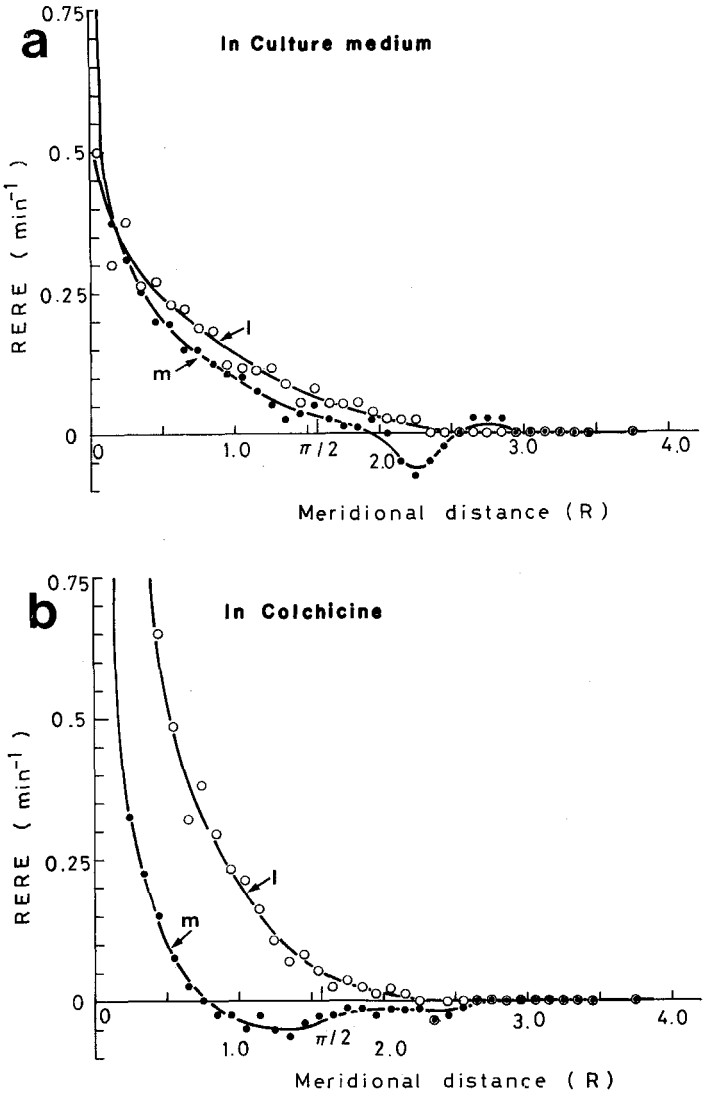

Fig. 6. REREs of normal and colchicine-treated apices. a: Normal apex. Meridional (m) and latitudinal (l) components of RERE are plotted against m. b: RERE of expanded apex in the presence of colchicine (2×10^{-4}M, from 2 days before on).

(Fig. 6b) at around 1.0 R suggest a slight shrinkage in longitudinal direction at the region.

Orientation of cell wall microfibrils

Polarized light microscopy revealed that the alga's cell wall specimen was positively birefringent. Since the chemical composition of the microfibrils of *Vaucheria* cell wall has been identified as cellulose (Parker *et al.*, 1963), the positive birefringence means that the average orientation of microfibrils is parallel to the cell axis. On the other

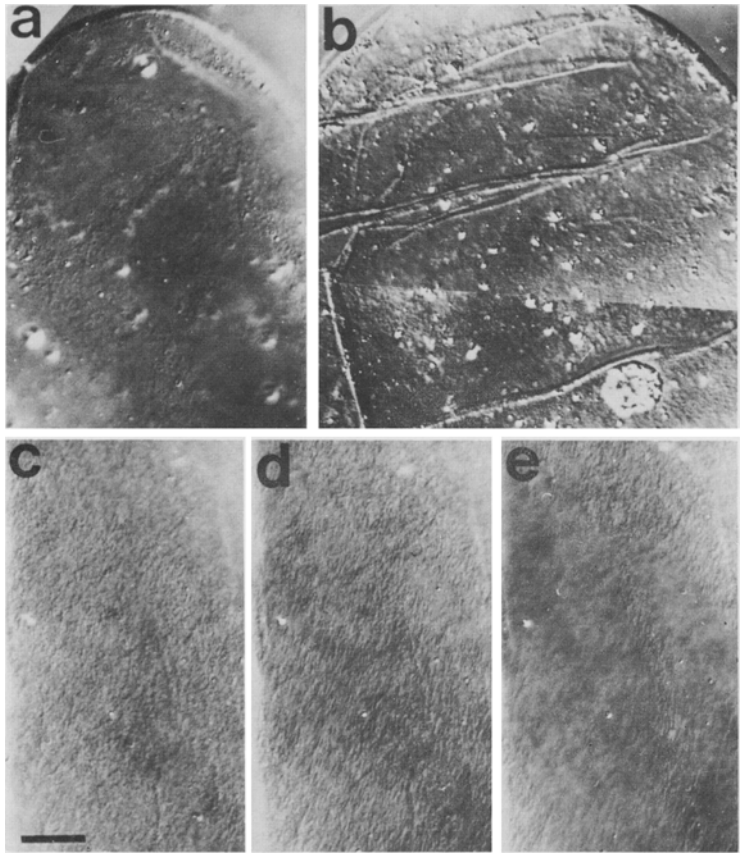

Fig. 7. Cell wall texture of *Vaucheria geminata* observed by Nomarksi optics. a: Normal apex. b: Colchicine-treated apex (2×10^{-4}M, 3 days treatment). c, d, and e: Optical sections of a single subapcial thickened cell wall specimen of non-treated cell; c, outermost, d, middle, and e, innermost layer. Bar indicates 10 μm.

hand, no clear optical anisotropy was detected at the apical dome region. This is either due to the random orientation of microfibrils, or to the cell wall at the actively growing apex being very thin (<0.3 μm). The fibrillar orientation can also be detected by high magnitude Nomarski optics (Fig. 7). A clear axially orientated texture can be seen at the basal cylindrical portion of the cell. Although the orientation in the outermost layer is rather random (Fig. 7c), that of the inner layer (d and e), is parallel to the cell axis. In the non-treated apex even a faint anisotropic orientation could not be detected (Fig. 7a). Contrarily, dim transversal orientation was observed at the basal half of the apical dome of colchicine-treated cell (Fig. 7b). The role of the transversal fibrous matter will be discussed in Discussion section.

Lack of effect on phototropic response of colchicine

Phototropic response of this alga cell is accomplished within the apical hyaline cap region (Kataoka, 1975a, b, 1979, 1981). A new growth center was initiated at the

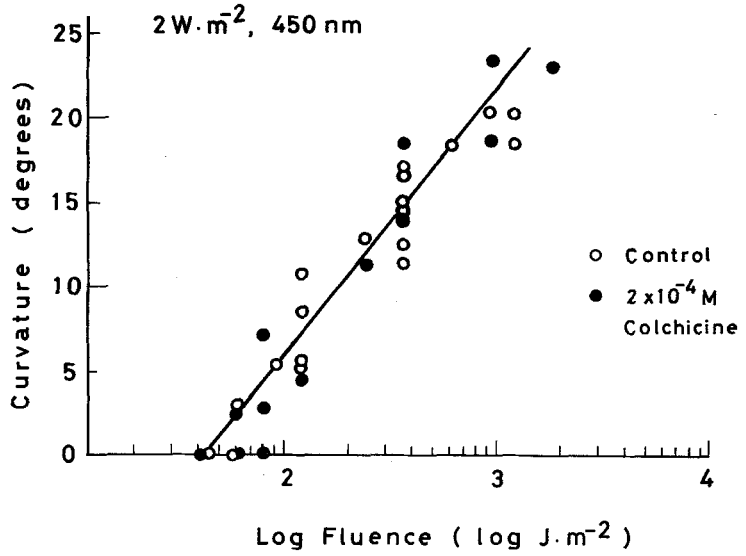

Fig. 8. Fluence-response curve for the first positive phototropic bending of colchicine-treated apices. Longitudinal halves of actively growing apices were irradiated with blue light (450 nm, 2 W m^{-2}) for various periods of time. Each data point is from a single measurement.

blue light-irradiated flank of the apical dome, and the original growth center decayed simultaneously. The symmetrical irradiation with strong blue light for longer than 5 min caused expansion at the apex (Kataoka, 1981). Thus, it is worthwhile to examine the interaction between light and colchicine.

Both control and colchicine-treated expanded apices were subjected to the half-side-irradiation with blue light (450 nm). Fig. 8 shows the fluence-response relationships, proving that the first positive bending is not affected by colchicine. This indicates that colchicine-induced redistribution of growth activity is independent of phototropic response. The morphological change of colchicine-induced apical expansion resembles the blue and red light-induced one (Kataoka, 1981), although the time-courses are somewhat different from each other. REREs have not yet been determined in the light-induced expansion phenomenon, because of the experimental difficulties. It will, however, be necessary to compare the kinetics of both expansion responses further.

Discussion

The tip-growth of *Vaucheria* is quantitatively demonstrated. Compared with some other tip-growing cells, such as young frond of *Bryopsis* or *Acetabularia*, apical cell of *Nitella* (Green, 1969), or young sporangiophore of *Phycomyces* (Castle, 1958), the geometry of *Vaucheria* cell apex is much closer to an exact hemisphere; the growth activity is almost limited to within this hemispherical dome. It was first found that the extension of cell wall (and/or cell membrane) of this alga is almost isotropic. If one draws a small circle on the flank of the dome near the apex, it will not be distorted during

its movement down towards the bottom of the dome. The kinetically proved isotropic surface expansion is consistent with the random orientation of cell wall texture at the apex.

The transversally oriented microfibrils have been found in many diffuse-growing cells such as pea stem parenchymatous cells (Veen, 1971) or *Nitella* internodal cells (Green, 1965; Gertel and Green, 1977). The occurrence of microtubules just beneath the plasmalemma running parallel to the wall microfibrils have also been well documented (Ledbetter and Porter, 1963; Pickett-Heaps, 1967; Newcomb, 1969; Hepler and Palevitz 1974). In such a diffuse-growing cylindrical cell, the synthesis and/or orientation of microfibrils take place transversally (Gertel and Green, 1977). On the other hand, the situation is quite different in tip-growing cells such as root hairs, pollen tubes, fungal hyphae, fern protonemes, and some alga cells like *Vaucheria*. At the growing apical dome microfibrils are randomely oriented, while at the cylindrical part below the dome, they are almost axial (Newcomb, 1969). Chen (1980) demonstrated the positive birefringence of cell wall in *Nitella* rhizoid. This is also the case in *V. geminata*. The axially running microfibrils seem to be analogous to the secondary thickened cell wall of fiber cells. Predominant axial texture at the non-growing basal part of the cell is supposed to be strong enough to stand the stress along the hoop direction, which is twice as large as that along the cell axis. If the turgor was reduced, the cell would not be as shrunken as was the *Nitella* internodal cell or the parenchymatous cell of *Avena* coleoptile. A precise measurement of mechanical properties of cell wall of this alga is, however, required for further discussion.

As for the apical region, the random cell wall texture seems to be consistent with the hemispherical cell shape, and with its isotropic pattern of growth. On the other hand, the relationship is not so clear in the colchicine-treated cell. Although colchicine at high concentration ($>10^{-3}$M) caused irreversible cessation of growth, at moderate concentrations ($\simeq 2\times10^{-4}$ M) it brought about cell expansion without changing surface expansion rate. RERE*s* were determined by cells in which the longitudinal growth and expansion were balanced dynamically. In such a case, one could not distinguish a colchicine-treated cell from a control cell without determining the growth kinetics. Note that the hemispherical shape of the apical dome can be maintained not only by isotropic surface expansion but also by anisotropic expansion. According to Green and King (1966), the transversally anisotropic growth requires that the wall of the basal half of the dome is always stiffer, or mechanically stronger, than that of the apical half. This can be easily understood, since a larger dm/dt value at small m is the condition of transversal anisotropy (Fig. 5). The faint transversally running fibrous structure found in the basal half of colchicine-treated apex (Fig. 7b) might serve as the mechanical reinforcement especially along the hoop direction.

However, there is an alternative possibility. Namely, the stiffness at the base does not necessarily mean a thicker wall or deposition of microfibrils along the hoop direction at the base. A thinner or weaker cell wall at the distal half of the dome will also result in a relatively stiff wall at the base. If this is true, the transversal fibrous

material is merely a by-product of expansion or of longitudinal shrinkage (Fig. 6b). Taking into consideration that the fibrous materials are scanty, the latter possibility may be much more plausible.

A preliminary electronmicroscopical study revealed that the apical transparent region (hyaline cap, Kataoka, 1975a, b) was composed of closely packed vesicles. Inasmuch as the presence and the size of this structure is correlated with the alga's growth activity, and inasmuch as this structure shifted to the blue light-irradiated flank prior to the phototropic bending being detectable, the vesicles no doubt act in an essential role for tip-growth. They must fuse with plasmalemma and extrude wall material by a mechanism of so-called exocytosis. As shown in Fig. 6, surface area expansion rate (*m-RERE*+*l-RERE*) reaches 100% min^{-1} at the very apex. This extremely high rate is 720 times and 60 times as high as that of *Nitella axillaris* leaf apical cell (Green and King, 1966), and that of protonematal cell of *Pteridium aquilinum* (Davis, 1975), respectively. Thus, the rate of fusion between vesicles and plasmalemma will be expected to be very high. A minute and transient change of hyaline cap distribution will lead to direct and instantaneous morphological change. The phototropic bending is initiated by rapid movement of the hyaline cap which occurs within 2 min of irradiation (Kataoka, 1975a, b).

Recently, Howard and Aist (1980) demonstrated that in hyphal tip cells of *Fusarium acuminatum* an antimicrotubular reagent, methyl benzimidazol-2-ylcarbamate, stopped the cell growth, by preventing vesicle transport towards the apex. This drug did not cause apical expansion of this fungal cell, but modified the distribution of vesicles (Spitzenkörper) and cut the supply of vesicles. Colchicine was not effective in the case of this fungus (Howard and Aist, 1980). A similar mechanism could be, however, anticipated in *Vaucheria* cell apex treated with colchicine. When the longitudinal growth and lateral expansion are balanced by the moderate action of colchicine (2×10^{-4} M), both growth activity (*m-RERE*+*l-RERE*) and hyaline cap region are shifted to the distal half of the dome (see Fig. 4b and Fig. 6b). The fact that the rate of surface extension does not change during the colchicine-treatment (Fig. 3) suggests that the volume of hyaline cap remains unchanged. Upon treatment with colchicine, the hyaline cap, the pack of vesicles, will be spread to some extent. This leads to broadening of growth zone and expansion at the apex. However, as the effect is not so strong as to completely destroy the established polarity, the longitudinal growth and expansion will reach a dynamic equilibrium. The rapid restoration of the original cell diameter when colchicine is washed out (Fig. 3) may indicate microtubules, if present, are rapidly turned over as suggested by Howard and Aist. A great many more cytological and physiological studies at the subcellular level are needed to determine any relationship between anisotropic wall synthesis and change in kinetics or distribution of vesicular fusion in such a tip-growing cell.

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