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Phototropic bending and light-induced branching in <u>Vaucheria</u> <u>geminata</u>.

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分枝 の 誘導.）

Hironao Kataoka

Phototropic bending and light-induced branching

in Vaucheria geminata

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A xanthophycean coenocytic alga Vaucheria geminata exhibited a typical tip-growth. The growth pattern was closely related to the external L-D regime. Under 12L-12D regime, the alga started to grow at the beginning of the dark period and ceased to grow at the end of the light period; while under 24L-0D, it grew with a constant rate ranging from 100 to 200 μm/hr. The alga also exhibited a positive phototropism. Using half-side-illumination method the mechanism of phototropic bending and branching in Vaucheria geminata was analyzed. The bending process followed the Weber-Fechner's law and Bunsen-Roscoe's law. The action spectrum for the phototropism was determined, and the result indicated that only blue light was effective. It was shown that the phototropic bending was initiated by the formation of a new growth center on the illuminated side of the apical dome of the tube, and not by the difference in growth rate between the lighted and shaded sides of the tip. The hyaline cap, which was found only at the growing tip, shifted to the illuminated side of the tip prior to the bending.

Abbreviations: L, light; D, dark; xL-yD, x hr L and y hr D.

Branching was also initiated when the part proximal to the apical dome was illuminated. Blue light was effective for the branching as it was in the case of phototropic bending. The refractive indices measured at the growing tip and the area other than the tip were 1.36 and 1.34 respectively. A hyaline patch resembling to the hyaline cap, which appeared prior to the onset of tip-growth in the apical dome, was invariably formed shortly before the initiation of a new branch at the illuminated locus.

Introduction

The plant body of <u>Vaucheria geminata</u> is a sparcely branched coencytic tube. Without giving detailed kinetics Klebs (<u>1</u>) reported that the growth of this alga was by mean of tip-growth. The phototropism in <u>Vaucheria</u> was described for the first time by Oltmanns (<u>2</u>). He found that at a moderate light intensity the growing tube bent toward the light, that at a very high intensity, away from the light, and that under a given intensity, the sign of phototropism did not change whether the alga was in water or in a moist chamber. According to Weber (<u>3</u>), light with wavelengths shorter than those of red light was effective for phototropism in <u>Vaucheria</u>. There have been few detailed studies on the phototropism of tip-growing cells, but most of them dealt with either fungal hyphae (<u>4</u>) and sporangiophores (<u>5</u>, <u>6</u>), or with fern protonemes (<u>7</u>).

The mechanism of phototropic bending in the fungal germ tube was studied extensively by Gettkandt (<u>4</u>). Using Buder's half-side-illumination method, she demonstrated that in the germ

tubes of <u>Puccinia</u> <u>triticina</u> and of some related species the growth
in the shaded side was greater than in the lighted side. From
this fact she concluded that this negative light-growth reaction
was the cause of the negative phototropism under unilateral
illumination, which converged the light on the surface of the
tube away from the light. The phototropism in mature sporangio-
phores of <u>Pilobolus</u> <u>kleinii</u> is also caused by differential growth
(<u>5</u>, <u>8</u>). In the young sporangiophores of this fungus however,
Page (<u>5</u>) found that a quite different mechanism was operating
in phototropic bending. Before formation of the sporangium, the
sporangiophore of <u>Pilobolus</u> showed the tip-growth as in the case
of <u>Phycomyces</u> (<u>9</u>). According to Page, a new growth center was
formed on the illuminated side near the original tip of the
young sporangiophore with a simultaneous decay of the original
growth center, and hence the new tip grew toward the light.
This mechanism was found also in polarotropism of a fern
<u>Dryopteris</u> (<u>7</u>). Haupt (<u>10</u>) mentioned that the formation of a new
growth center should be common to all tip-growing cells in photo-
tropism. Illuminating a small locus on the flank near the very
tip of the young sporangiophore of <u>Pilobolus</u>, however, Page and
Curry (<u>6</u>) admitted later that the differential growth also
participated in the phototropism of the sporangiophore when it is
young. Green <u>et</u> <u>al</u>. (<u>11</u>) showed a schematic diagram of the bending
mechanism in tip-growth. There they drew a sharp line between
these two types of mechanisms, namely the differential growth and
the formation of a new growth center. They named the first type
"bowing" and the second, "bulging". In this paper it will be shown
that the tip of <u>Vaucheria</u> bends toward the light by forming a new
growth center on the illuminated side of the growing apical region.

Material and methods

Culture conditions

The alga was collected in 1970 at Albany, New York State, U.S.A. by Dr. N. Kamiya. The alga was identified as Vaucheria geminata (Vauch.) De Condolle. var. geminata by Dr. H. Hirose of Kobé University (cf. 12). Contaminated organisms were eliminated by pulling the plant covered with charcoal powder in gelatin (Ohiwa, personal communication). The culture solution used was a combination of Uspenski's (cf. 13) and Darden's (14) media with some modifications. The following ingredients were dissolved in distilled water to make 1000 ml of final volume: 25 mg KNO_3, 25 mg $MgSO_4 \cdot 7H_2O$, 100 mg $Ca(NO_3)_2 \cdot 4H_2O$, 13.6 mg KH_2PO_4, 582 μg $MnCl_2 \cdot 4H_2O$, 30 μg $CoCl_2 \cdot 6H_2O$, 24 μg $NaMoO_4$, 4.5 mg Na_2-EDTA, 0.0001 μg Biotin and 0.0001 μg Vitamin B_{12}. The medium was buffered (pH = 7.2) with 1 mM Tris-HCl, then it was autoclaved at 120°C for 20 min before use. The alga was cultured in the growth cabinet under 12 hr light and 12 hr dark (12L-12D regime) at 20°C. The light intensity was 1000-1500 lux, from white fluorescent tubes (Toshiba FLR20 S-W/M). For the experiments of phototropism, the alga was cultured for 3 days under continuous light at about 1000 lux at 20°C in 6 cm petri dishes, in order to nullify the circadian rhythm of growth (see later pages). The alga having the growth rate of about 200 μm/hr was used except otherwise stated.

Staining with calcofluor

In order to stain the cell wall, a fluorescent dye Calcofluor White ST (American Cyanamide Co. Bound Brook, N.J.) was used. This dye is well known by its affinity with cell walls of many

plants (15). A small portion of the thalli having several growing tips was incubated for 30 min at 20°C in 0.05% Calcofluor solution buffered with 10 mM Tris-HCl at pH 7.2. Then the alga was carefully washed 5 times with the buffer. The tips which continued to grow were observed under a fluorescent microscope 2 hr after the treatment.

Resin adhesion

Green (16) reported that particles of weak anion exchanging resin adhered well to the cell to the cell surface of Nitella. In the present experiments Dowex A-1 (Cl$^-$-type) beads were changed to OH$^-$-type with NaOH, washed well with distilled water, and ground in a glass homogenizer. Tiny resin particles with diameters of 1-10 μm were obtained by centrifugation. A drop of the resin suspension was added to the petri dish in which a few thalli with several growing tips were immersed in the culture medium. The dish was shaked gently so that the particles adhered on the thalli, then a cover slip was placed on them to hold the material in position.

Optics employed

Experiments were carried out on the stage of a microscope in a dark room at 20°C. Fig. 1 shows the diagram of the optics illuminating locally a small region of the tube of the alga with two beams of monochromatic light. The light sources (C_1 and C_2) were low voltage incandescent lamps with lens (Olympus Microscope Co. Ltd.). A glass plate (M), 5 cm × 5 cm, was made from Kodack slide mounting glass. One half of it was made to a surface mirror with vacuum evaporation of aluminium,
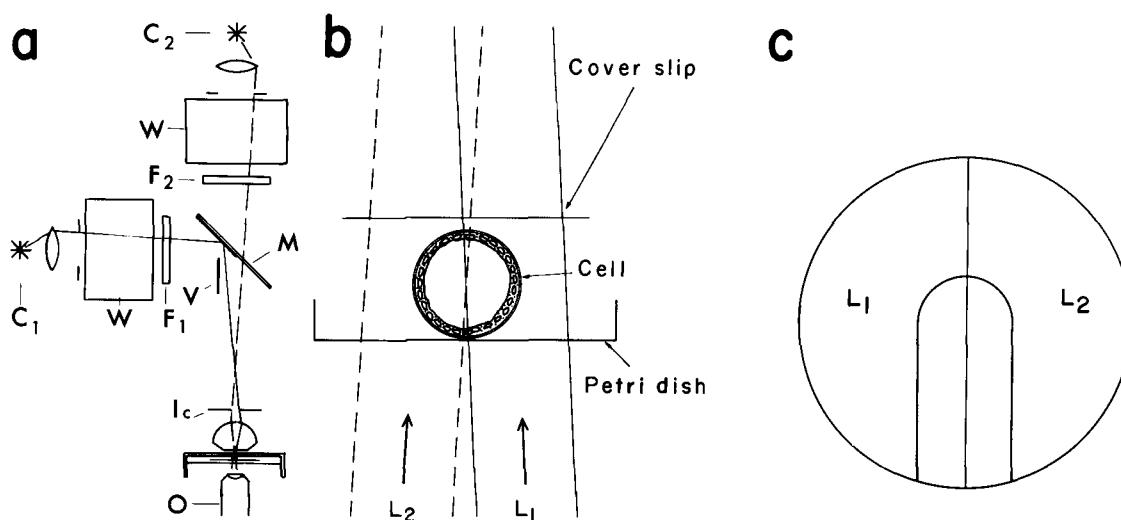
Fig. 1   Schematic diagram of experimental setup.

(a) The optics employed; (b) two monochromatic beams, comming from a small condenser aperture, and going through the cell; and (c) the half-side-illumination in the microscopic field. The mirror of the microscope is omitted in the diagram (a). For further details, see text.

and the other half, left transparent. The plate was set at 45° against the optical axis of the microscope. With this setup the light beam from C_1 reflected at the mirror half of the plate and the light beam from C_2 passed through the transparent half of the plate so that the two beams reached the right and left fields of the microscope, respectively. In order to eliminate the reflected light at the plain glass surface, a black vane (V) was put in front of M (Fig. 1a). The images of the two lamp filaments were focused on the condenser aperture; and the image of the edge of the mirror (M) was focused on the alga in a petri dish placed on the stage of the microscope (Fig. 1b). The heat generated from the lamps was reduced by water containing glass cell (W) placed in front of the lamps. Desired monochromatic light beams were obtained by using interference filters (Toshiba KL-series) (F_1 and F_2), which were placed between the glass cell and the plate (M), as shown in Fig. 1a. To eliminate further the heat entirely, heat cutting filters (Toshiba IRQ-80) were placed next to the interference filters; and to eliminate excess secondary interfered light, if necessary, a glass filter (Toshiba color glass filter AT-series) was also placed between the glass plate and the interference filter. Thus a light spot could be obtained, of which one half was occupied with a monochromatic light from F_2. This, being a Köhler illumination, produced the two light beams illuminating the sample as shown in Fig. 1b, when the condenser aperture was small.

For unilateral illumination, a cubic glass chamber containing the alga placed on the stage of a microscope was illuminated with a beam of actinic monochromatic light horizontally, i.e., the beam reaching the alga parallel to the plane of the

microscope stage. Red light needed for the observation of the material was given through the condenser of the microscope.

Measurement of light energy

The energy of the light spot was determined on the microscope stage. The area of the spot was smaller than the smallest area measurable with thermopile radiometer (Kipp & Zonen), which can indicate the measured light intensity directly in the unit of $ergs \cdot cm^{-2} \cdot sec^{-1}$. Thus the light intensity in terms of voltage of the spot was first measured with silicon solar cell (Hayakawa Electrics Co. Ltd., SBC-510), which made the measurement of such a small spot possible. Then a simulation of a bigger illuminated area with the intensity equal to the measured voltage was made, and the bigger area was measured with the thermopile radiometer to get $ergs \cdot cm^{-2} \cdot sec^{-1}$ expression.

Phototropic bending

Prior to the experiments, the alga cultured for 3 days under continuous white light was moved to the dark and left for at least 1 hr. To unify the growth rate of experimental material, the alga which grew at the rate of about 200 μm/hr was used. To induce phototropic bending a longitudinal half of the apical region was illuminated with actinic blue light (450 nm) and the other half with red light (673 nm) for observation (cf. 17; Fig. 1).

The phototropic bending was expressed with the angle (θ) between the original cell axis and a new cell axis. The both cell axes were determined in the following ways (see Fig. 6,

upper drawing): On the enlarged photographic print an arc (B_0 or B), whose center (A_0 or A) was at the center of the hyaline cap (H) and whose radius was that of the hemispherical dome, was drawn; the axis was then constructed by drawing a line running through A_0 or A and the middle point (M_0 or M) of the chord ($B_0 C_0$ or BC).

Action spectrum

The action spectrum was determined with the compensation method. The longitudinal half of the growing tip was illuminated with the reference light (479 nm) with the intensity equal to 1050 ergs·cm^{-2}·sec^{-1} and the other half, with various monochromatic lights of various intensities. The balancing light intensity where the tip bent neither toward the reference half nor test half was determined after several trials of 15 min's illumination. The intensities thus obtained were expressed in the unit of quantum flux density (pE = 10^{-12} Einstein). The reciprocals of the ratios between these values and the similar value of the reference light (420 pE) were plotted against wavelengths.

Phototropic sensitivity of the tip

To compare the local sensitivity to the light in the tip region, the basal region of the longitudinal half of the growing tip (B in the inset of Fig. 10) was illuminated with blue light (450 nm, 1000 ergs·cm^{-2}·sec^{-1}) for 15 min and the curvature was measured immediately after the illumination. The vertical distance (x) from the front edge of the beam of the base of the dome is expressed as a fraction of the radius of the dome (R).

Measurement of refractive index

To determine the refractive index of the growing apical region occupied with protoplasm and that of the basal region occupied mostly with the large central vacuole, a transmitted-light interference microscope (Leitz, Wetzler) was used. The amount of shifting in interference fringes caused by the material was measured and the refractive index was calculated with the following equation:

$$n_c = \frac{D \cdot \lambda}{d \cdot t} + n_w,$$

where n_c and n_w are indices of the cell and the surrounding medium (= 1.333) respectively; d, distance between fringes; D, an amount of shift (in a unit of wavelength) of the fringe when the material was inserted; t, actual thickness of the material, and λ, the wavelength of the used monochromatic light (= 546 nm).

Results

Characteristics of growth in Vaucheria

a) Growth pattern

Fig. 2 shows the growth of Vaucheria under 0L-24D, 12L-12D, and 24L-0D regimes with the light intensity of light periods being at about 1500 lux. The optimum growth of the alga occurred under the 12L-12D regime at 20°C. Under 0L-24D, the growth took place only during the first 24 hr. Under 12L-12D regime, the majority of growth activity was in the dark period; it grew well during the dark period and gradually ceased to grow in the
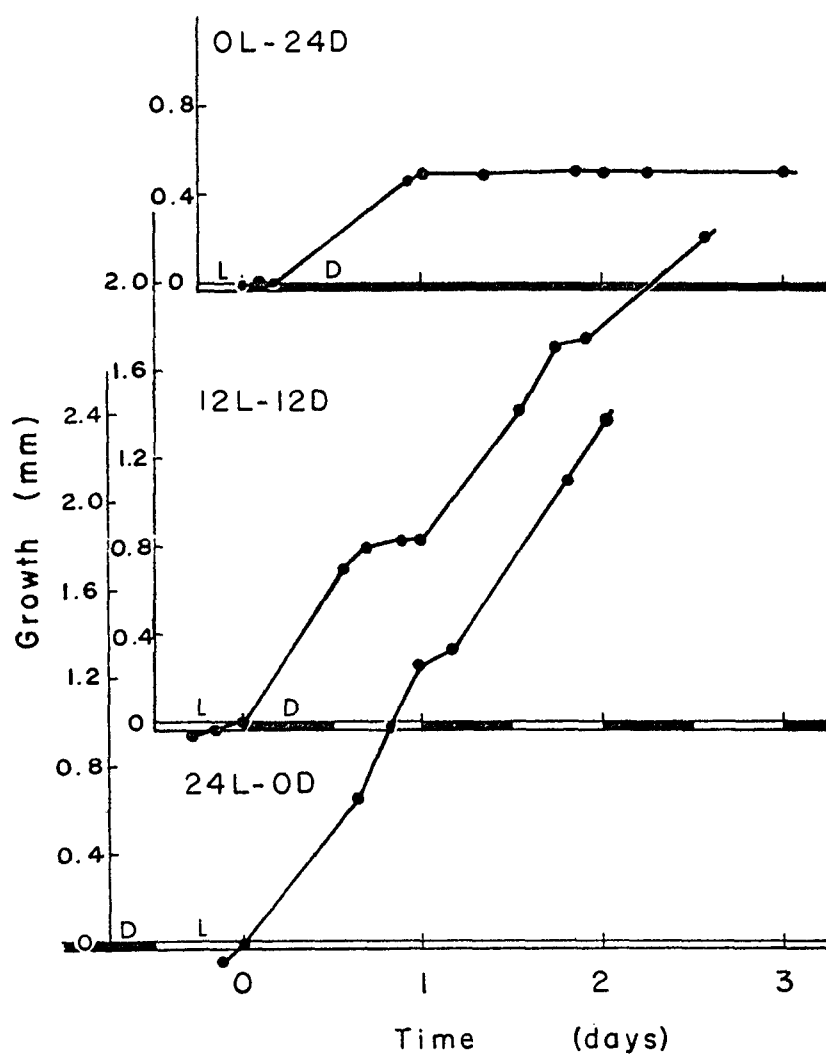
Fig. 2   Rhythmic growth under 12L-12D regime, the
cessation of growth under OL-24D and the disappearance
of the rhythm under 24L-OD. Each plot represents an
average values of 11 tubes under 12L-12D, 9 under OL-
24D, and 26 under 24L-OD. Alga was cultured at 20°C
and the light intensity of the light period at 1120 lux.

light period, reaching to no growth at the end of the light period. When the alga was transferred from 12L-12D to 24L-0D (continuous light), the periodicity in growth rate was retained for 24 hr following the transferring and then it grew surprisingly at a constant rate for 5 to 7 days. That a morphological change occurred when the alga started to grow at the beginning of the dark period under 12L-12D regime is obvious from Fig. 3. This diagram was obtained by tracing a series of photomicrographs. As shown in the figure, the demarcation was formed when the growth took place again. The cell wall at the apical region seemed to become thicker during the cessation of growth and formed a thick-walled apical dome. The constriction part of the demarcation was the result of restarting of the growth, in which the newly grown part originated first from a restricted apical portion of the thick-walled dome (Fig. 3, upper inset), then temporary increased its diameter somewhat. The demarcation thus formed served as a reference point with which the growth was measured. The fact that the distances between all of the two adjacent demarcations were equal to one another under 12L-12D regime means that in L-D regime the growth rate was kept constant for many days.

b) Tip-growth

As shown in Fig. 4, there was no Calcofluor stain at the apical region. This is a good evidence demonstrating that the newly growth region of the tip was composed only of the newly synthesized cell wall. The tip-growth phenomenon is further supported by Fig. 5 which shows the resin particles attached to the alga as index markers, and that no growth was observed
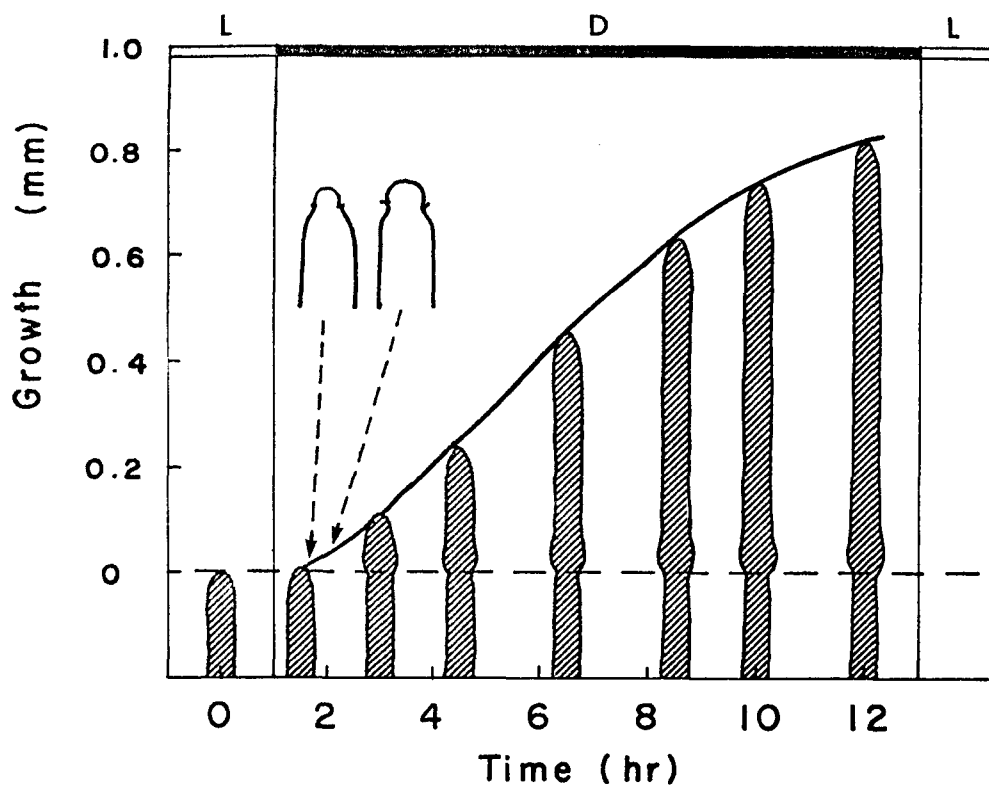
Fig. 3   Diagram showing the rate of the growth and the
formation of a demarcation resulted from restarting of
growth at the beginning of D-period under 12L-12D
regime. The profiles of a tube were obtained by tracing
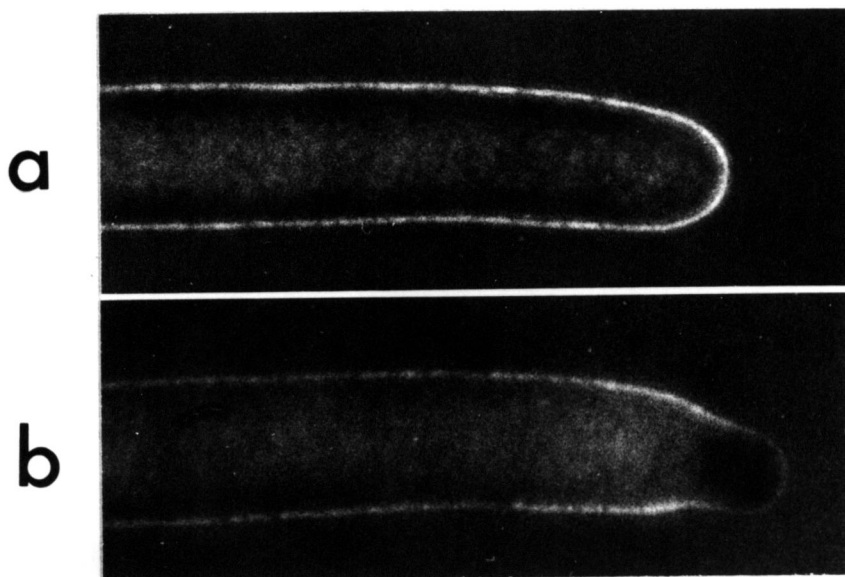photomicrographic prints.

Fig. 4   Vaucheria stained with Calcofluor White ST, and observed under fluorescent microscope. (a) The alga stained uniformally after the free dye was washed out; (b) unstained apical region appeared 2 hr after the staining, showing the synthesis of cell wall at that region after washing.
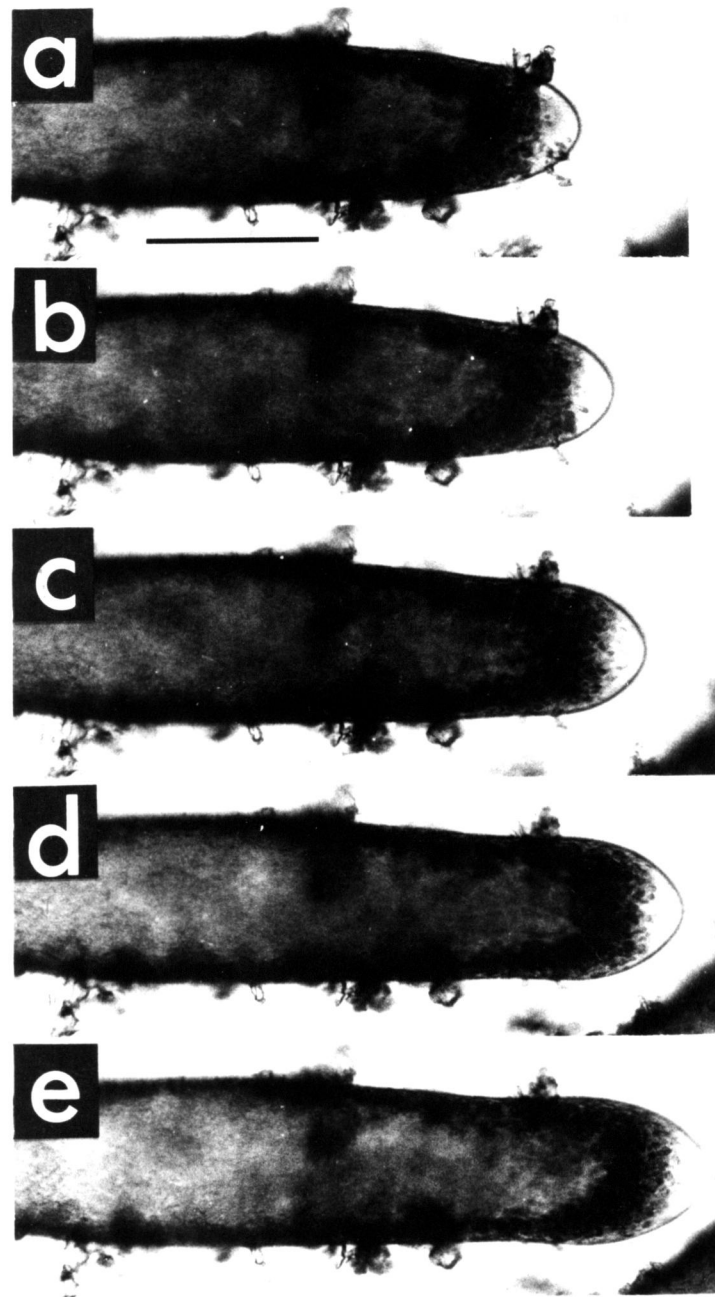
Fig. 5   Series of photomicrographs showing a growing tip with resin particles adhered. A clear hyaline cap is visible. The photographs a, b, c, d, e, were taken at 5 min intervals. The line indicates 0.1 mm.

behind the hemispherical dome of the apex. Proximal to the cell wall at the apex, a hyaline cap, from which the chloroplasts were completely excluded, was formed as far as 15 μm from the very apex. Since the hyaline cap was observed only during the growth period, its presence could be used as a criterion of active growth.

Phototropic bending

a) Time-course of bending

From Fig. 6 it is clear that the bending started 1 to 2 min after the onset of half-side-illumination with blue light, increased rapidly during the illumination (from time zero to the periods of time indicated with arrows), and continued slowly for 13-20 min after the light being turned off. The final curvatures seemed to be proportional to the logarithms of the illumination period, with an exception of 30 min illumination.

b) Dose-response relations

Fig. 7 shows that the curvature, measured 900 sec after the beginning of illumination, increases proportionally to the logarithm of the illumination period at a given intensity, when the longitudinal half of the tip was illuminated. In each intensity the proportionality starts from some threshold energy value, which is the product of duration and the intensity. For example when the alga was illuminated with light of 450 nm at 1000 ergs$\cdot$cm$^{-2}\cdot$sec^{-1}, it needed the illumination of slightly longer than 40 sec to cause the bending. Therefore, the figure demonstrates that the curvatures below 26° follow the Weber-Fechner's law. The Fig. 7 also indicates that the higher the
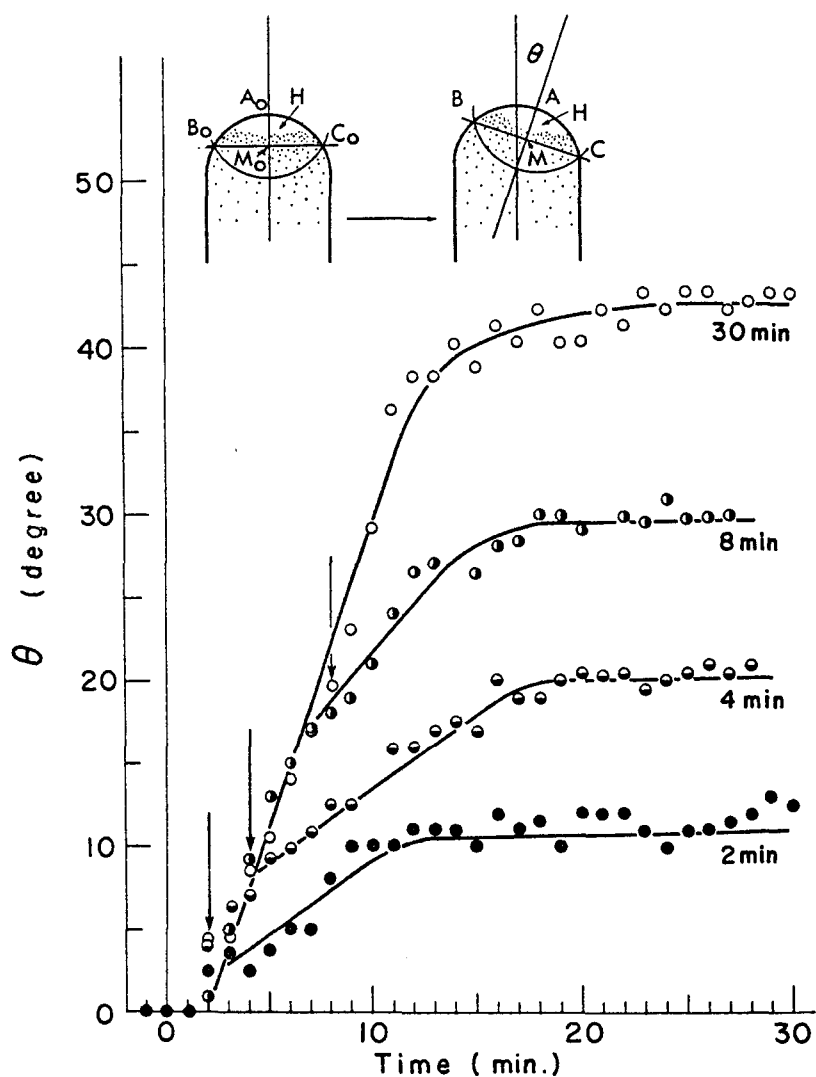
Fig. 6   Plots of phototropic bending vs. time observed
under various period of half-side-illumination.
Arrows from left to right respectively indicate the
time when the blue light was turned off at 2, 4 and 8
min after the beginning of illumination. The top curve
was obtained with continuous half-side-illumination for
30 min. Actinic light used was 450 nm, 1300 $ergs \cdot cm^{-2} \cdot sec^{-1}$,
and observation light, 630 nm, 1000 $ergs \cdot cm^{-2} \cdot sec^{-1}$.
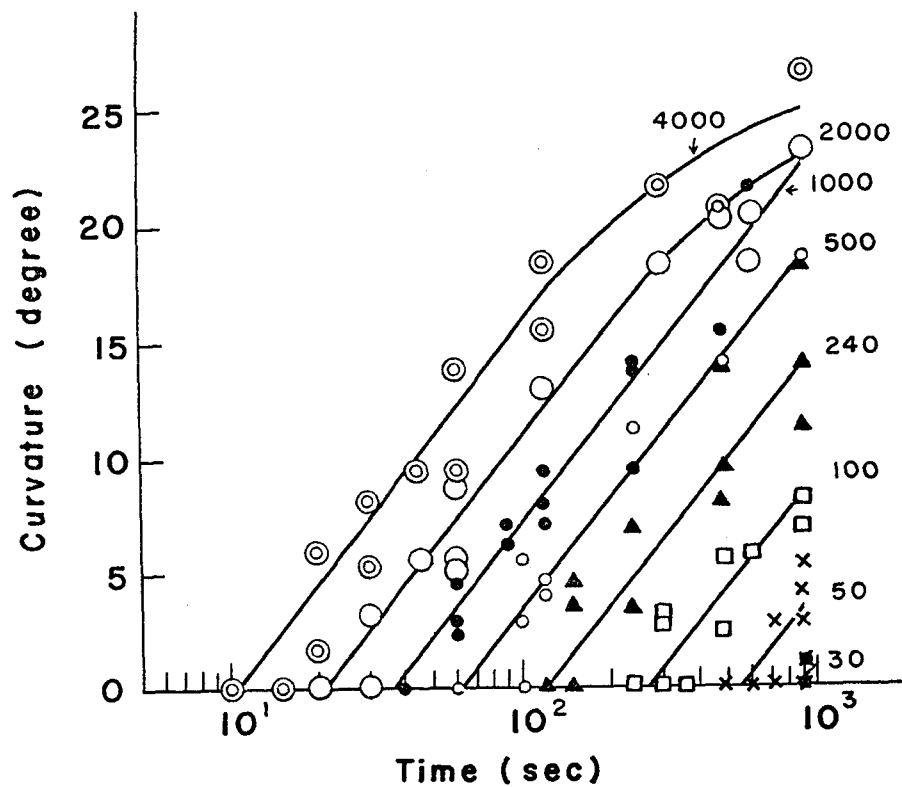
Fig. 7   Relationships between periods of half-side-
         illumination and the curvatures of bending. Growing
         tips were half-side-illuminated for varied periods from
         zero up to 900 sec, then kept in the dark, and the
         curvatures were measured 900 sec after the beginning
         of illumination with different light intensities.
         Numerals on the right hand of the respective curves
         indicate the energy (ergs$\cdot$cm$^{-2}\cdot$sec^{-1}) of actinic light,
         450 nm. Each plot is a value from a single measurement.

energy, the shorter the duration of illumination to induce the onset of bending. Such relationship is numerically demonstrated in Fig. 8 in which the light energy was plotted against threshold times read from Fig. 7. The higher the intensity (I), the shorter the time (T). It is clear from Fig. 8 that the amount of stimuli (I × T) for causing the onset of bending is constant (about 3×10^4 ergs$\cdot$cm^{-2}). Thus the phototropic bending in Vaucheria follows the Bunsen-Roscoe's law.

c) Action spectrum

The action spectrum of the phototropism is plotted in Fig. 9. The quantum efficiency at 450 nm is highest and it diminishes as the wavelength becomes shorter or longer. When the wavelength is longer than 550 nm the efficiency reaches nearly to zero.

Branching induced by blue light

Illuminating a small region of the plant with the blue light (450 nm, 1000 ergs$\cdot$cm$^{-2}\cdot$sec^{-1}), which is effective for the phototropic bending of the growing tip of this alga, also induces branching from the portion of the tube other than the tip, as shown in Fig. 10. Immediately after the onset of illumination, the cytoplasm together with many chloroplasts accumulated gradually at the lighted portion. This accumulation continued as long as the alga was kept illuminated. After 1 hr's illumination, the thickness of the cytoplasmic layer reached 10 times of the initial thickness. After about 4-5 hr's illumination a small hyaline patch appeared just underneath the cell wall (Fig. 10c) at about the center of the lighted area. Then a protuberance was formed from the patch 30-60 min after the
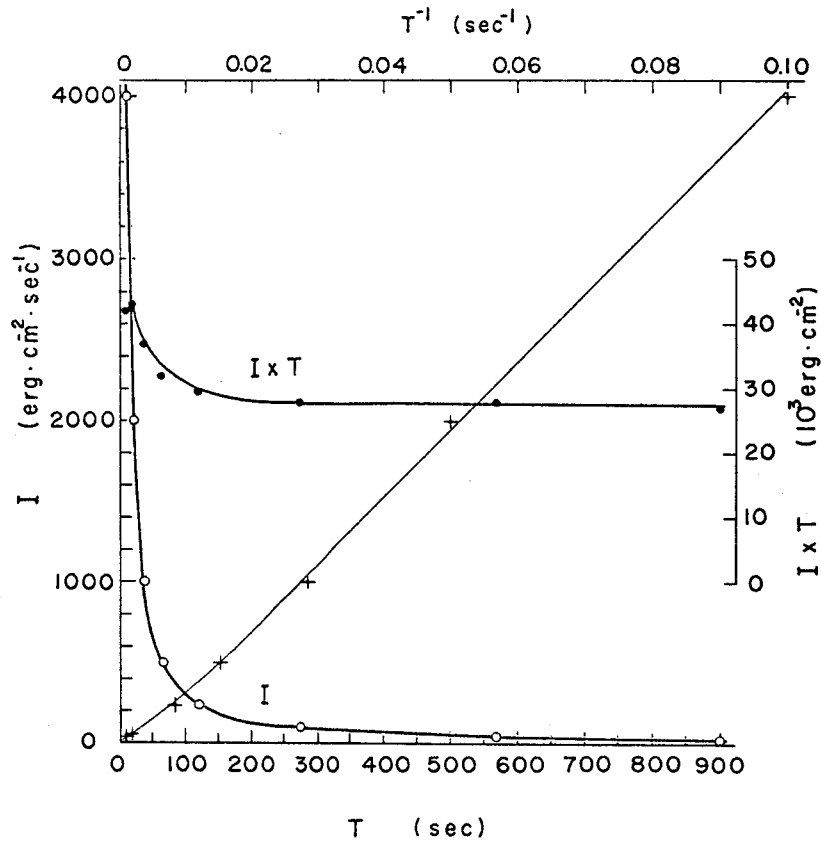
Fig. 8   Relationships between intensities of light (I)

and illumination period required for threshold bending

(T).   I- and T-values are obtained from Fig. 7.   I-

values are plotted against T- and against T^{-1}-values.

Abscissas of the coordinates represented by circles

(o and •), and that of the coordinates represented by

by +-markes (+) are respectively the lower and upper

horizontal lines.   The vertical scale at the right is
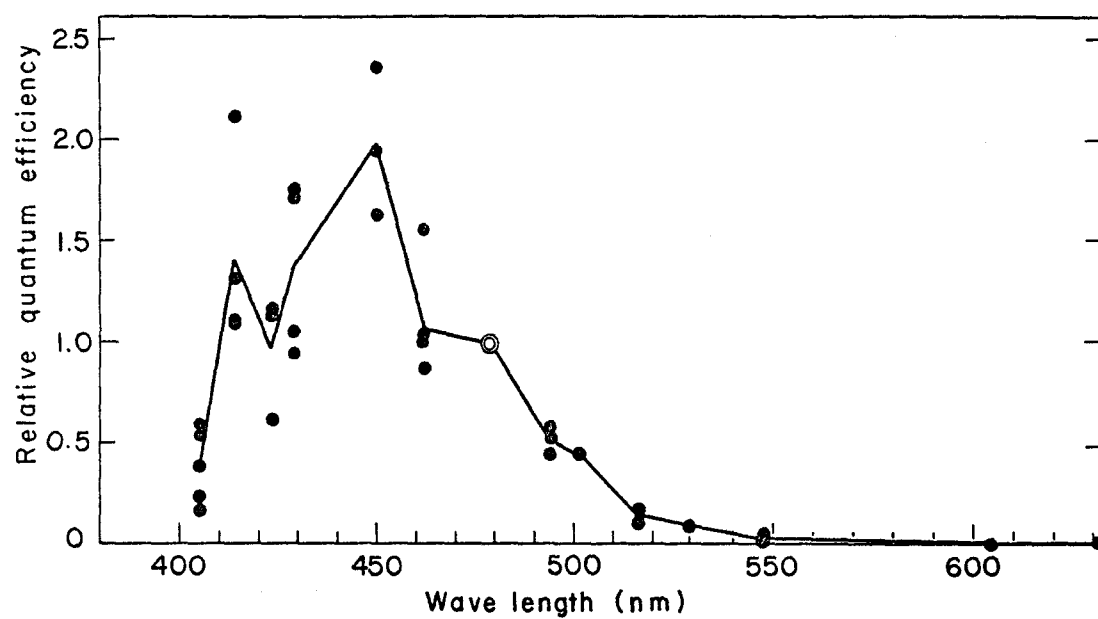
the ordinate for I × T plots.

Fig. 9   Action spectrum for the phototropic bending of
Vaucheria geminata. Each plot represents a datum of
a single plant. Lines are connecting the average
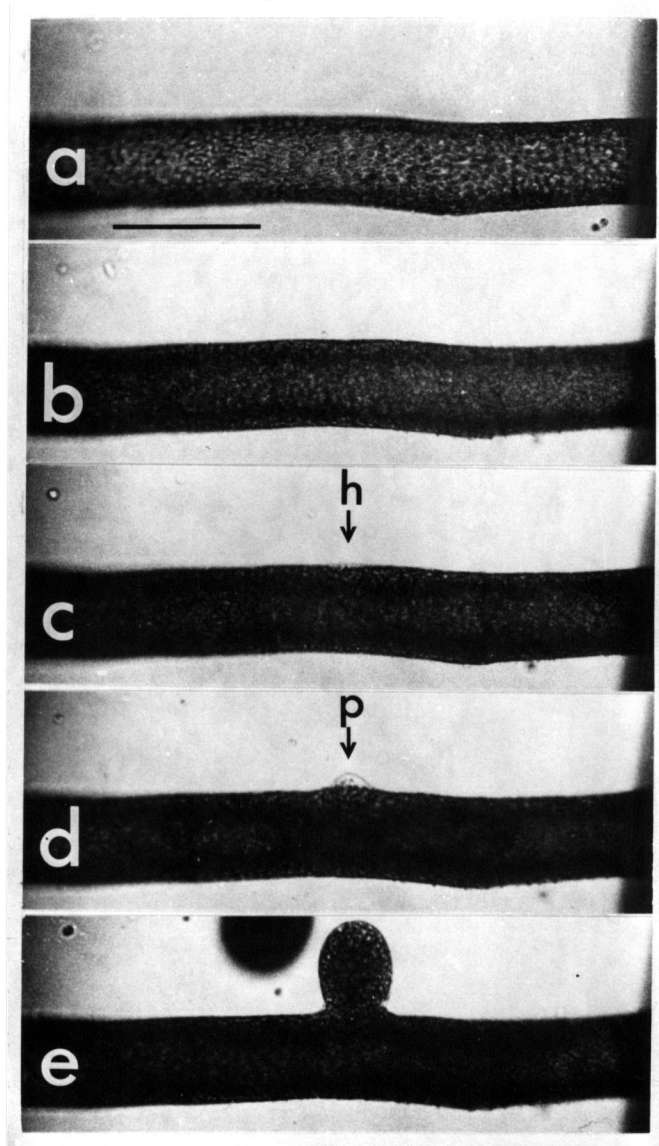values of data obtained under each wave length.

Fig. 10   Microphotographs showing branch formation by local illumination with blue light (450 nm, 1000 ergs·cm^{-2}·sec^{-1}). Illumination area was 0.41 mm in width. Pictures in series were taken at 0 (a), 3 (b), 4.3 (c), 6.7 (d) and 7.5 hr (e) after the illumination. h: a hyaline patch; p: a protuberance. Scale 0.1 mm.

appearance of the patch (Fig. 10d), and it grew into a branch (Fig. 10e). At least 2 hr's illumination with energy at 1000 ergs·cm^{-2}·sec^{-1} and wavelength at 450 nm were necessary for the induction of branching. It took at least 5 hr for the alga to develop a recognizable protuberance, no matter whether the tube was irradiated continuously for the 5-hr period or only for the first 2 hr in the 5-hr period. The light with wavelength longer than 550 nm had no effect on the induction of the branching. For instance, continuous illumination of 673 nm light at 5 × 10^3 ergs·cm^{-2}·sec^{-1} for 96 hr resulted no branching. The range of wavelength effective for branching was essentially the same as that for phototropic bending, i.e., between 400 and 520 nm. The hyaline patch which appeared just before branching seemed to be homologous to the hyaline cap located in the growing tip of the tube.

Mechanism of phototropic bending

a) Bulging process

In the diagram of Green et al. (11), the mechanisms of the phototropic bending are classified into two types. One type, named "bowing", is a differential growth, and the other, named "bulging", is the formation of a new growth center or migration of the growth center. To determine which one between the two mechanisms, i.e., bowing and bulging, is involved in the phototropic bending in Vaucheria, a tiny resin particle was attached onto the growing tip. In case the bending is caused by the "bowing" mechanism under the condition of half-side-illumination, a small resin particle adhering to the very tip (Fig. 11A,

particle a) would not leave from the tip throughout the course of bending. On the other hand, in case the bending is caused by the "bulging" mechanism (Fig. 11B), the resin particle adhering to the very tip would be displaced from the tip toward the base on the dark side; because a new growth center should be formed on the lighted side near the tip. However the resin particle, which adheres to the point of the presumptive new growth center (Fig. 11B, particle b), should remain at the center of the new growth center during the bending. Fig. 12 is a series of time-lapsed photographs taken during the early stage of bending. At the initial stage of half-side-illumination the apical hyaline cap migrated to the lighted side, and after 8 min of the illumination, reached the place where the center of the cap coincided with the presumptive center of the new growth. Thereafter, the particle, which stuck at the presumptive center, stayed at the same position respective to the new growth. This fact is accounted for only by assuming that the phototropic bending of this alga is by "bulging".

b) Phototropic sensitivity of the tip

In Vaucheria, only the apical region is phototropically sensitive. The bending did not occur unless x/R value (the distance between the base of the dome and the front edge of the beam expressed as a fraction of the radius of the dome) becomes greater than approximately 0.6, thus the only sensitive area to the light in the growing tip is apical half of the dome (Fig. 13). This area corresponds to the region occupied by the hyaline cap. This decrease in sensitivity along the cell axis may be partially due to the increase in number of chloroplasts which shade the light.
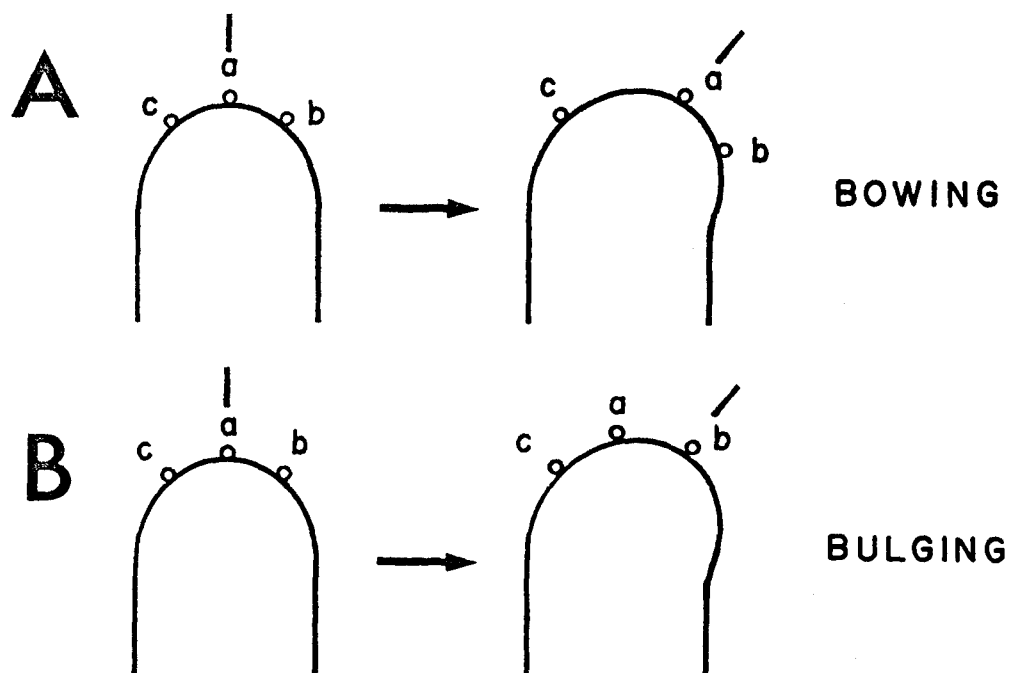
Fig. 11   Theoretical movements of index markers attached

to the cell walls, according to the mechanisms of

bowing and bulging in comparison. In bowing (A) the

distance between the two markers, a and c, would increase

due to the elongation in that side. In bulging (B) the

particle b which adhered on the center of the presump-

tive new growth center would remain at the new very

tip during the bending. Short lines indicate the axes
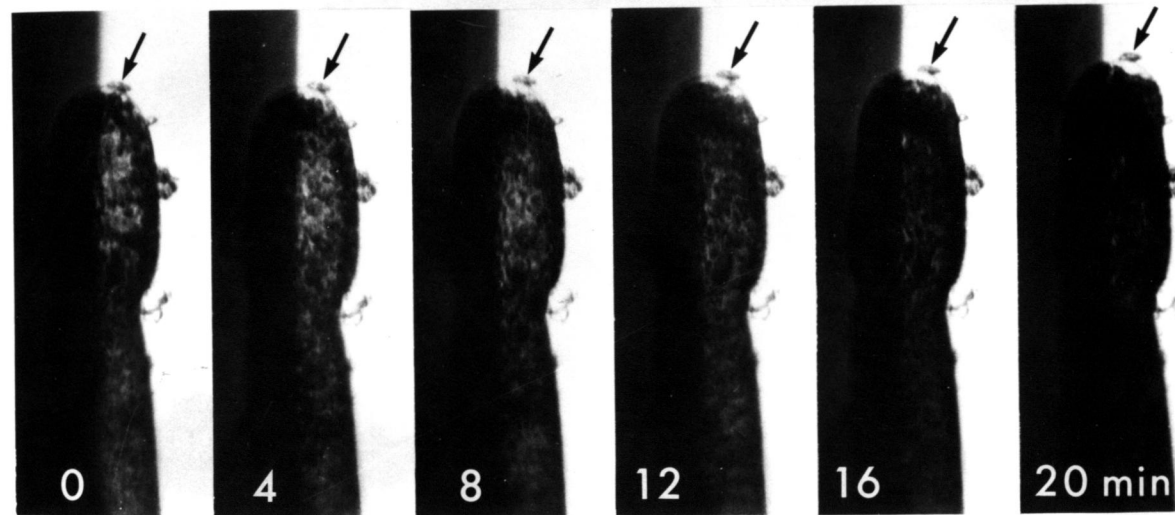
of straight growth and bending.

Fig. 12   A serial micrographs showing bulging process in phototropic bending. Arrows indicate an index marker corresponding to b-marker of the theoretical example of bulging type in Fig. 2. Right halves of pictures: illumination with 450 nm, 1000 ergs·cm^{-2}·sec^{-1}. Left halves of pictures: 673 nm, 300 ergs·cm^{-2}·sec^{-1}.
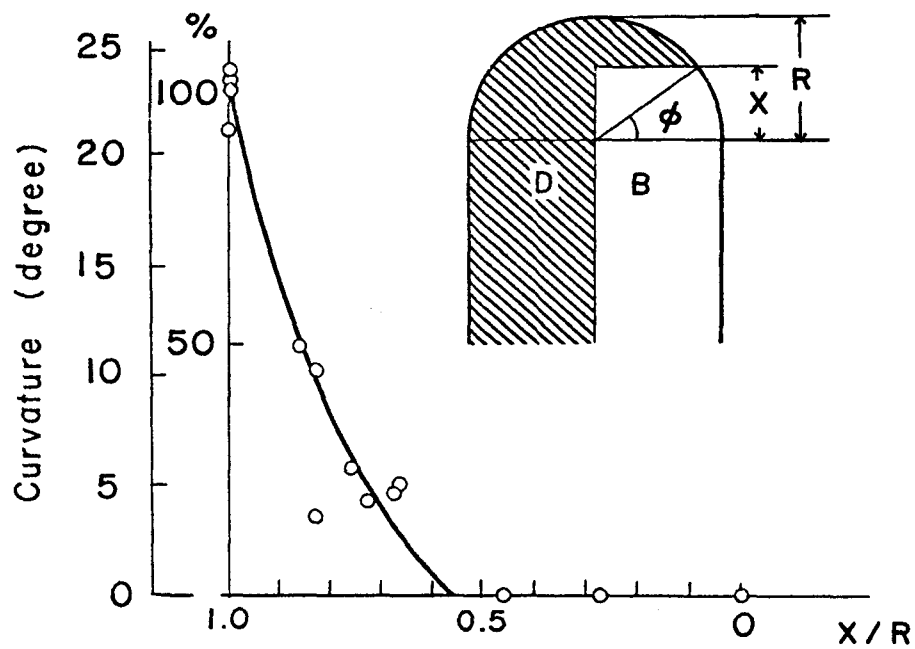
Fig. 13   Phototropic sensitivity along the cell axis, showing the closer the illuminated region to the tip of the dome, the greater the degree of bending. The extent of the illuminated area is represented by the region without hatching in the inset.

c) Light-growth reaction

In the field of half-side-illumination with blue light, the actinic wavelength of the phototropism, the tip bent toward the lighted side. If the bending is the result of the differential growth mechanism, we should be able to observe a higher growth rate on the dark side than on the lighted side. Namely, the alga should exhibit the negative light-growth reaction caused by the blue light. However, this possibility was excluded by the following experiment. The tip of the tube was first illminated with blue light (450 nm, 1000 $ergs \cdot cm^{-2} \cdot sec^{-1}$) for a certain period, then the same tip was illuminated for some period with red light (630 nm) which provided the same energy as that of blue light. The growth rates were always higher when the alga was illuminated with blue light than it was with red light. Two examples are shown in Fig. 14. Under blue light, whether the duration of the illumination was between 20 min to one and half hr (Fig. 14b), or as brief as 2 min (Fig. 14a), the growth rates were higher than the rates under red light illumination. Thus, it could be concluded that this alga shows a positive light-growth reaction when it is illuminated with blue light (cf. 18-20).

d) Unilateral illumination

The alga growing in moist air, culture solution, or liquid paraffin was illuminated unilaterally with blue light (479 nm, about 500 $ergs \cdot cm^{-2} \cdot sec^{-1}$). In all cases, in spite of being surrounded by media of different refractive indices, the alga exhibited positive phototropism, although the bending was weak.
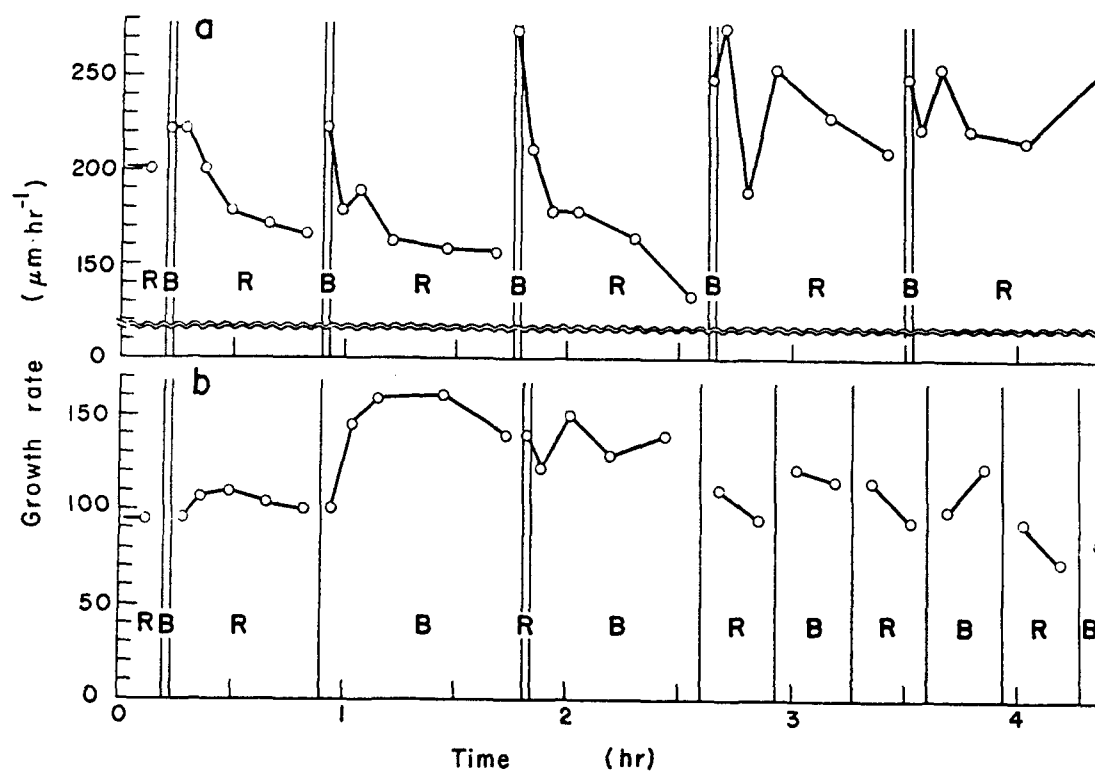
In order to find out why the sign of phototropism remained

Fig. 14   Enhancement of growth by blue light.   B represents
the duration of illumination with light of 450 nm at
1000 ergs·cm^{-2}·sec^{-1}.   R represents the duration of
illumination with light of 630 nm at 1000 ergs·cm^{-2}·sec^{-1}.
Notice that the growth rate is always higher in B-
period than in R-period.

positive irrespective of the great difference in refractive indices of the media (1.00 for air, 1.33 for water, and 1.47 for liquid paraffin), the refractive index of the growing tip was determined. The refractive index obtained with the transmitted-light interference microscope was 1.364 with standard error of 0.002 (number of measurements 24) for the growing tip, and 1.340 with standard error of 0.001 (number of measurements 4) elsewhere. The obtained refractive index of the tip is close to 1.38, the refractive index of mature sporangiophores in Phycomyces measured by Castle (21), but is significantly lower than 1.57 obtained by Page and Curry (6) in young sporangiophores of Pilobolus kleinii. Fig. 15 is a schematic diagram showing the light path through the growing tip, assuming the alga is grown in the air, the culture solution, or liquid paraffin. The refractive indices of the air, the culture solution and liquid paraffin are taken as 1.00, 1.33, and 1.47, respectively. When the alga is in the air, the incident light beam should be accumulated at the basal region of the hemispherical dome of the distal side. On the other hand in every case, as far as the first apical third of the dome is concerned, the drawings indicate that the side of the dome facing to the light receives more light than the distal side does. (Notice that the density of the lines is higher on the side facing to the light.) As described before (Fig. 13), the first apical third of the dome is the only region sensitive to the light for positive phototropic bending in this alga.
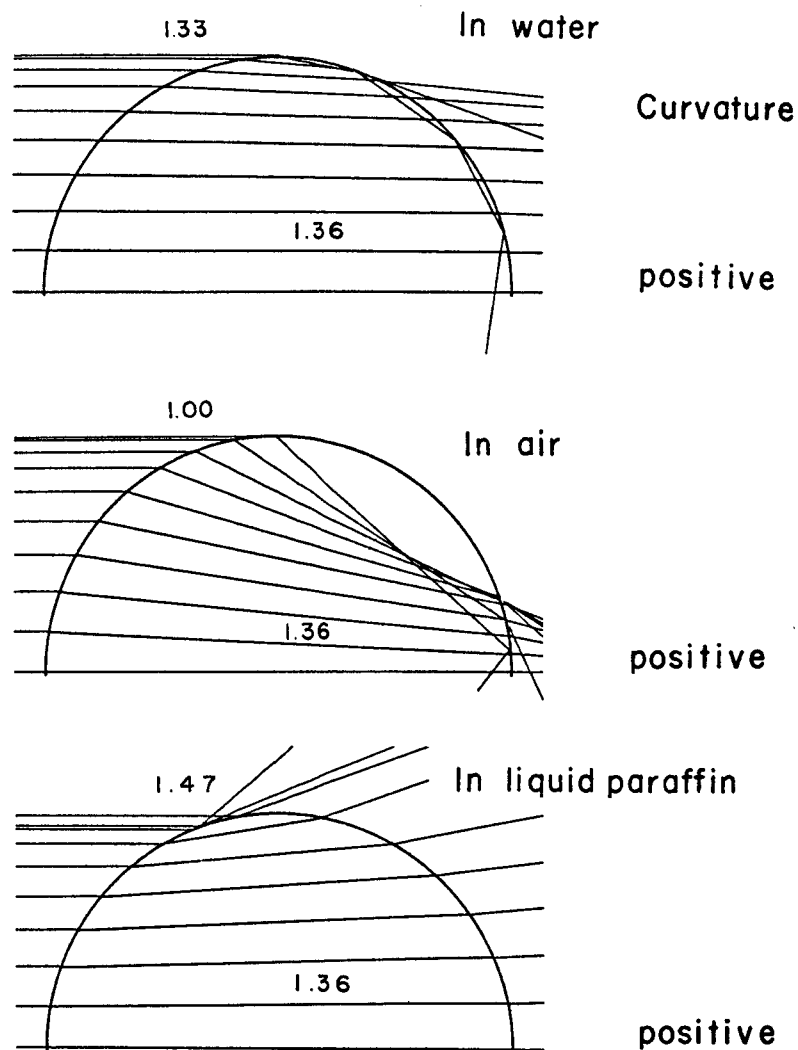
Fig. 15   Schematic diagram showing the light paths of
unilateral illumination through the apical dome of
Vaucheria in various media of different refractive
indeces, and the signs of phototropism when the tip
was under such illumination. In every case, as far
as the light-sensitive first apical third of the dome
is concerned, the side facing to the light (left-side)
receives more light than the distal side (right-side)
does. The observed phototropic bending was always
toward the incident light.

Discussion

Although the action spectrum obtained here lacks UV-region, the peaks at around 410, 450 and 480 nm are in good agreement with the peaks obtained for the first and second positive phototropism in Avena coleoptiles (22-24), and with peaks for the light-growth reaction and phototropism in mature sporangio-phores of Phycomyces (25-27). It is interesting to note that the action spectra of the phototropism in Vaucheria described in the present paper, of the chloroplast movement in Vaucheria (28), and of the photodinesis in Vallisneria (29) are all similar to one another. The chloroplast accumulation in Vaucheria was suggested (28, 30, 31) to be due to the light-induced increase in viscosity of the cytoplasm. Fisher-Arnold (28) found that in Vaucheria sessilis the chloroplast accumulation was promoted by the red light which was given after the blue light; however, the effect of red light was not cancelled by the subsequent far-red irradiation. In the present experiments red light did not promote the phototropic bending, excluding the possibility of phytochrome taking an active role in the bending.

In Vaucheria blue light not only caused phototropic bending at the tip of the tube, but also induced branching elsewhere on the tube. Similarity of the action spectra in the both phenomena suggests that the photoreceptor-system is the same for the both.

To study the location of the photoreceptor in the cytoplasm, the outermost flank of the apical dome was skimmed with the small light beam. Under such illumination, a phototropic bending occurred toward the lighted side. Thus, it is very likely that the photoreceptor(s) in Vaucheria resides in the outermost layer of cytoplasm as in Mougeotia (cf. 32-34). In this connection

it is to be added that phototropism and branch formation in Vaucheria could be induced also with polarized blue light irrespective of the direction of E-vector.

Based on the fact that a resin particle, which stuck on the lighted flank at the presumptive site of the new apex of the tube, became situated at the very apex of the new growth after the bending, it is sufficient to say that the bending is according to the bulging mechanism. Furthermore, the possibility of the bending due to the negative light-growth reaction, which if occurs would cause the tube to bend toward the lighted side, is excluded by the result mentioned in the paragraph of light-growth reaction in the Results section. And, since the bending is toward the lighted side, conventional positive light-growth reaction, which if occurs would cause the tube to bend away from the lighted side, is definitely not the mechanism. These leads to the bulging mechanis, the observed fact, being the kinetics of bending in Vaucheria.

When a unilateral illumination, instead of half-side-illumination, was applied on the tube surrounded by the air, water, and liquid paraffin, the tube bent toward the light in all three media. These could be explained by the geometrically constructed light path, indicating the first apical third of the dome receiving slightly more light on the surface facing to the light than its corresponding part of the distal surface. When the tube is surrounded by the air, the constructed light path, however, shows more light converged at the basal flank of the dome on the distal surface. Nevertheless the observed bending was toward the light. This could be explained by two observed facts: 1) The part sensitive to light for phototropic

response is localized only in the first apical third of the dome; and 2) the cell wall at the basal part of the dome is thicker than its apical dome (preliminary measurement of cell wall thickness with an electron microscope showed that the wall thickness at the first apical half of the dome is about 0.3 μm whereas it is 1 μm or more elsewhere of the tube).

It seems to be pertinent now to recapitulate important visual events leading to the bending and branching and consider them in comparison.

The phototropic bending: 1) When the longitudinal half of the growing tip is illuminated with blue light, the hyaline cap starts to shift (1-2 min after illumination) quickly to the lighted side and finally settles down (4-5 min after illumination) at the new position. The time necessary for the bending to become microscopically discernible is less than 6 min. 2) The bending continues until the axis of the direction of new growth coincides with the midline of the settled hyaline cap. This fact indicates clearly that the growth center is situated at the center of the hyaline cap.

The branching: 1) When a small portion (0.5 mm) of the tube other than the tip is illuminated with blue light, a hyaline patch appears underneath the cell wall at the center of the illuminated area about 4-6 hr after the illumination. Then, a protuberance develops from the hyaline patch to form a branch. (When a relatively broader portion of the tube, for example, a few mm, is illuminated, more than one branches are formed.) 2) To induce the branching, it needs at least 1.5-2 hr's illumination even with light of high intensity.

Comparing the phototropic bending and branching, two

differences are noticeable. Namely, in branching, both the amount of stimuli and the reaction time needed are exceedingly larger than in phototropic bending. Since the thickness of the cell wall is 0.3 μm around the tip, while it is 1 μm or more elsewhere, the thickened cell wall at the region proximal to the tip may account for the necessity of a higher intensity and a longer period of illumination for the induction of branching. Preliminary observations with an electron microscope reveal that the hyaline cap is filled with many vesicles containing fibrous materials. These fibrous materials are probably cell wall precursors; if it is so, their existence in the fast growing tip is understandable. In the case of branching, these vesicles probably could also be found in the hyaline patch formed at the site of presumptive branching. Based on the characteristics common to both phototropic bending and branching in Vaucheria, it could be concluded that blue light increases the extensibility of cell wall and/or promotes the excretion of cell wall materials at the illuminated region.

At the tip, where the cell wall is thin, a small increase in the wall extensibility at the illuminated side will inevitably result in a rapid formation of a hump which then develops into the new tip. For the protuberance formation, since the cell wall in the region other than the tip is thick, the required long exposure probably is causing the cell wall to be extensive enough at least at the initial stage of the induction. The fact that the blue light induced some change in cell wall texture (increasing the extensibility and/or decreasing the thickness) is supported by the following observation. When the tube in early phase of bending (4-6 min after the onset of

half-side-illumination) was pressed strongly, the tube bursted at the flank of the apical dome where the future tip is to be developed. Whereas if a similar press was applied on a normal tip, it made the burst at the very apex of the dome, indicating that in normal tip the cell wall at the flank is thicker than the very apex. The role of vesicles in phototropic bending and in branching in Vaucheria will be published elsewhere. Positive phototropism and branching in rhizomes of the marine alga Caulerpa scaraliformis were also induced by blue light (unpublished).

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