Planta (1988) 173:490–499

Planta

Blue light promotes ionic current influx at the growing apex of *Vaucheria terrestris*

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Abstract. Irradiation of the growing apex of the alga *Vaucheria terrestris* Götz var. *terrestris* with blue light (BL), which causes a transient acceleration of growth, also causes a large transient increase in inwardly directed current, which was monitored with a vibrating probe. The growing apex is normally the site of an inward current, and the surface of the non-growing, basal part of the coenocytic cell the site of an outward current. Irradiation of the apex causes only a slight increase in current efflux at the basal part of the cell. The BL-promoted current influx at the apex (BLCI) usually starts within 10 s after the onset of irradiation, preceding the light-growth response. With BL pulses shorter than 3 min, the BLCI reaches a maximum in about 3 min, and then declines to its original value over the next 3 min. If the BL pulse is longer than 3 min, the BLCI continues until the light is turned off. The threshold energy of the BLCI with broad-band BL is 2–5 $J \cdot m^{-2}$, i.e. smaller than for both the light-growth response and phototropic response. The maximum BLCI reaches a value of approx. 5 $\mu A \cdot cm^{-2}$, equivalent to an influx of 50 $pmol \cdot cm^{-2} \cdot s^{-1}$ of monovalent cations. The effect of red light (RL) is completely different from that of BL: it either causes increases in the inward current of less than 0.3 $\mu A \cdot cm^{-2}$, or a transient decrease of current. Furthermore, the direction of the RL-induced change is always the same at the apex and trunk, indicating the participation of photosynthesis. Our results indicate that the BLCI is kinetically and spatially related to the light-growth response and the phototropic bending of *Vaucheria*. It seems to be a necessary step for the phototropic bending.

Key words: Blue light – Ionic current – Light-growth response – Phototropism (*Vaucheria*) – *Vaucheria* – Vibrating probe – Xanthophyta.

Introduction

Ionic currents are correlated with growth and differentiation in many plant and animal cells (see reviews by Jaffe 1980; Jaffe and Nuccitelli 1977; Weisenseel and Kicherer 1981). For instance, in tip-growing cells, such as *Pelvetia* zygotes (Nuccitelli and Jaffe 1974; Nuccitelli 1978; Jaffe and Nuccitelli 1977), lily pollen tubes (Weisenseel and Jaffe 1976), hyphae of *Achlya* (Kropf et al. 1984), and *Vaucheria* (Blatt et al. 1981; Kicherer 1985; Weisenseel and Kicherer 1981), these ionic currents always enter at the growing apex or other presumptive sites of growth and leave at non-growing, basal regions.

It is well known that in *Vaucheria* and many other tip-growing algae blue light (BL) is effective in eliciting a phototropic response, a modification of growth rate, polarity, branching, the movement of chloroplasts and other intracellular organelles (Kataoka 1975a, b, 1981; for reviews see Dennison 1979; Pohl and Russo 1984; Haupt and Wagner 1984). Ionic currents may, therefore, play an important role in the phototropic response of *Vaucheria*.

Recently, two investigations have demonstrated changes in the ionic-current pattern in *Vaucheria* which precede BL-induced responses. First, Blatt et al. (1981) found in *V. sessilis* that the irradiation of proximal regions of the tube with BL causes a transient outward current from the irradiated locus prior to the accumulation of chloroplasts in that region. From the kinetics of this re-

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Abbreviations: APW = artificial pond water; BL = blue light; BLCI = blue-light-induced current influx; LGR = light-growth response; RL = red light

sponse it was concluded that this BL-induced local current efflux must be a very early step in the aggregation of chloroplasts. Moreover, Blatt et al. (1981) assumed that the efflux might be a result of activation of proton pumps at the BL-irradiated plasmalemma. Second, it was demonstrated by Kicherer (1985), studying BL-induced branching, that the BL-induced local current efflux started to decay 3–4 min after the beginning of BL-irradiation. At about 60 min, when the accumulation of chloroplasts was completed, the outward current was replaced by gradually increasing inward current. Since the branching was always observed at sites of current entry, the onset of inward current apparently corresponded to the development of a new growth center at the BL-irradiated site.

The initiation of branching is similar to the phototropic bending at the apex. First, both processes comprise activation of local growth, although there are large temporal differences. Second, at the actively growing apex of *Vaucheria* there is a transparent cap region (Kataoka 1975a, b), which is formed by a massive accumulation of exocytotic vesicles (Ott and Brown 1974), and a similar transparent patch forms at the BL-irradiated, presumptive site of branching (Kataoka 1975b).

If an apex is unilaterally irradiated with BL or, if a longitudinal half is irradiated, the transparent cap shifts rapidly towards the source of BL, or the BL-irradiated side, and this leads to bending (Kataoka 1975a, b, 1981). The shift of the transparent cap can be detected microscopically within 2 min after the onset of BL. A local irradiation of the proximal region of the hemispherical apical dome with BL does not produce the shift of the apical cap (Kataoka 1981).

If the apex is irradiated uniformly with BL, a positive light-growth response (LGR), i.e., a transient acceleration of growth, occurs (Kataoka 1975a, 1987). The LGR starts after a lag period of approx. 1 min and reaches a maximum about 3 min after the onset of BL. The growth rate then decreases again to the pre-light level, or frequently undershoots it for 2–3 min, and often shows a damped oscillation with a period of about 10 min (Kataoka 1987). If the BL pulse is shorter than 2 min, the amplitude of the LGR obeys the reciprocity law. When the pulse is longer than 5 min, another type of growth promotion, i.e., the apical expansion becomes superimposed on the LGR (Kataoka 1981). However, since the apical expansion develops gradually and has a different wavelength dependency (i.e., red light can also cause the expansion, although its effectiveness is 1/500 lower than that of BL), it can be distinguished from the positive LGR.

The LGR is very sensitive to the pH of the medium: the threshold fluence at pH 7 is two orders of magnitude lower than that at pH 5 (Kataoka 1987). From close similarities in wavelength dependency, fluence-response relationship, and pH dependency, the hypothesis was proposed that a positive LGR is the primary necessary step of the positive phototropic response of *Vaucheria* (Kataoka 1987).

The aim of the present study was to search for changes of the ionic currents brought about by BL irradiation of the growing apex of *V. terrestris* and to correlate them with the LGR and phototropic response. *Vaucheria terrestris* was used for this investigation because this species is more sensitive to light than other *Vaucheria* species. Since insertion of a conventional glass microelectrode directly into the growing apex was impractical, because the apex is extremely sensitive to injury, we used the highly sensitive vibrating-electrode technique (Jaffe and Nuccitelli 1974; Dorn and Weisenseel 1982) to measure ionic currents extracellularly.

Material and methods

Alga. Vaucheria terrestris sensu Götz var. *terrestris*, the same strain as used in two previous investigations (Kataoka 1981, 1987), was used in all experiments. The alga was grown in artificial medium (Kataoka 1987) under white light (Osram-L 40 W; approx. 6 $W \cdot m^{-2}$) in a daily 12-h light/12-h darkness regime, at $17 \pm 2°$ C. Three or four days prior to an experiment, the alga was transferred to a 5-cm-diameter plastic Petri dish, the bottom of which had a rectangular window sealed by a piece of cover glass. The alga was fastened, by means of cooled 1% purified agar (Difco Laboratories, Detroit, Mich., USA), to a step made from a piece of cover slip on the margin of the window, covered with culture medium, and brought back to the culture room. The medium was replaced by artificial pond water (APW) 18 h prior to measurements. The APW contained 0.1 mM KCl, 0.5 mM NaCl and 0.1 mM $CaCl_2$, and was buffered with 1 mM 2-(N-morpholino)ethanesulfonic acid (Mes) or 1 mM 3-(N-morpholino)propanesulfonic acid (Mops) to the desired pH. The dish was then placed in darkness.

Electrical measurements. The dish was transferred to the vibrating-probe room 3 h before measurements. This room was kept at $20 \pm 2°$ C and lined with metal plates to shield it from external electromagnetic waves. The bathing solution was renewed twice, at 3 h and at 30 min prior to measurements, under dim (<0.1 $W \cdot m^{-2}$) green light (interference filter AL; Schott & Gen., Mainz, FRG; $\lambda_{max} = 546$ nm).

Extracellular electrical currents were measured with a vibrating probe (Jaffe and Nuccitelli 1974; Dorn and Weisenseel 1982). The apparatus used in the present experiments was almost identical to the one used by Blatt et al. (1981) and Kicherer (1985). The vibrating probe consisted of a metal-filled glass micropipet with a spherical platinum black ball of approx. 20 μm in diameter at the tip. The grounded reference electrode,

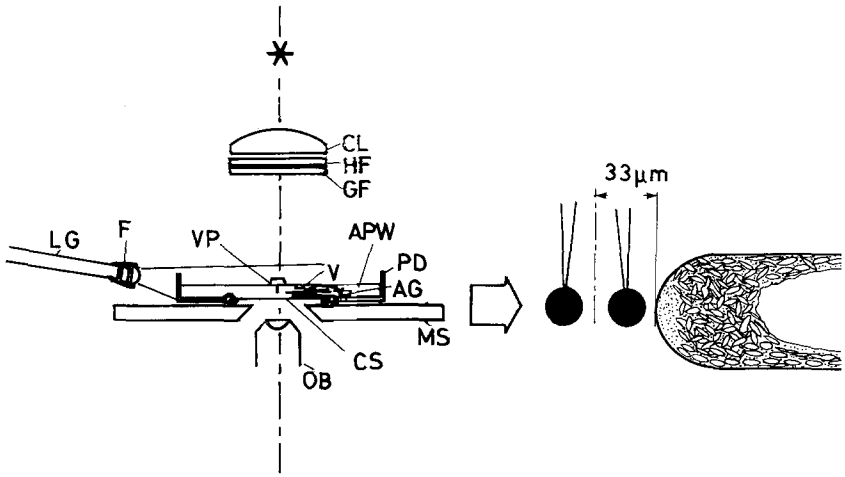

Fig. 1. Diagrams showing the experimental setup (*left*) and the geometrical arrangement of the vibrating probe at the apex of *Vaucheria* (*right*). *CD*, *HF*, *GF*, *MS*, and *OB* are, in this order, the condenser lens of the microscope illuminator, heat cut filter, green interference filter, rotating stage of the microscope, and the objective; *AG*, *V*, *VP*, *PD*, *CS*, and *APW* are agar drop, *Vaucheria*, vibrating probe, Petri dish, cover-slip window at the bottom of the dish, and artificial pond water; *LG* is the light guide; and *F* is a piece of plastic filter for the actinic BL or RL. Thalli 7–8 mm in length which grew straight and horizontally were selected for the experiments. The diagram on the *right* shows, at the same scale, the two extreme positions of the electrode tip and the algal apex. The direction of irradiation is from in front of the apex (*open arrow*)

made of platinum black, was electroplated as a collar onto the distal part of the pipet. The probe was mounted on a rotating stage above an inverted microscope (Invertoskop D; Zeiss, Oberkochen, FRG). The probe vibrated in a horizontal plane at a frequency of approx. 460 Hz and was driven by a piezoelectric element powered by the boosted oscillator output from a lock-in amplifier (Model 122; Princeton Applied Research Corp., Princeton, N.J., USA). The AC voltage from the vibrating electrode tip (V_{eff}) was measured by means of a preamplifier (Model 112; Princeton Applied Research) and the lock-in amplifier, and recorded on a chart recorder. The time constant of the lock-in amplifier was set to 0.3 to 1.0 s. The actual peak-to-peak voltage between the two positions (V_{pp}), equal to $2\sqrt{2}$, was converted to J, the local current density along the axis of vibration, according to Ohm's Law:

$$J=\frac{2\sqrt{2}V_{eff}}{\rho \cdot d}$$

where ρ is the specific resistivity of the bathing solution ($\Omega \cdot$cm), and d is the distance between the two extremes of the probe's center (cm), which was set at 33 µm. The specific resistivity of the solution was measured with a conductivity bridge (Model RC 21682; Beckman Instruments, Fullerton, Cal., USA) just before and immediately after each series of measurements. The ρ-value of APW 7 was about 7500 $\Omega \cdot$cm; V_{eff} and J are expressed as µV and µA$\cdot$cm^{-2}, respectively.

The arrangement of the probe, the cells, and the light sources is shown in Fig. 1. Horizontally growing apices of the alga were used for the electrical measurements. The axis of the probe's vibration was brought in line with that of the cell. The current was measured by placing the probe in front of the algal apex with its center of vibration at a distance of 33 µm from the surface of the apex. Because of the rapid growth, the distance of the probe relative to the apex was carefully adjusted to keep the value of 33 µm constant, by moving the x–y stage of the probe every 4–6 min. In this paper, the electric current (i.e., the flow of positive charge) is defined as the difference of J values at the measuring position and at the reference position, which was located approx. 300 µm away from the apex, i.e. at a site of apparently no current.

Light sources and treatments. For the actinic BL or RL, a light-guide-equipped quartz iodine lamp source (MKL 200; Muster, Adelsdorf, FRG) was used. Blue or red plastic filters (3 mm; Röhm, Darmstadt, FRG) were inserted between the end of the light-guide and the small convex lens. The fluence rates of the broad-band BL and RL at the cell surface were approx. 7 W$\cdot$m^{-2} and 50 W$\cdot$m^{-2}, respectively. This broad-band BL was sufficient for evoking a phototropic response, a LGR and a BL-induced current influx. A phototropic bending was not induced by this strong broad-band RL. As shown in Fig. 1, the light source of the actinic light was set to be parallel to the cell axis, and approx. 10° above the horizon at a distance of 5 or 7 cm.

Dim green light (<0.1 W$\cdot$m^{-2}) for watching the positions and movements of the probe and for observation of the alga was obtained with the microscope illuminator and interference filters AL 554 and KG 1 (Schott & Gen.) All measurements hereafter stated as being done "in darkness" were done in this dim green light. The alga's growth rate always decreased to some extent during the first 10 min of measurements, but then proceeded at a constant rate. This decrease is probably the result of convection of the medium causing some disturbance of the sensitive apex. Dim green light is unlikely to contribute to this initial retardation of growth because it has been observed previously (Kataoka 1975a, 1981) that the growth of *Vaucheria* is either not affected by such green light, or slightly promoted. All measurements reported in this paper were started about 10 min after the vibrating probe was placed in front of the alga's apex.

Chemicals. Mes, Mops, $CaCl_2$, KCl, NaCl and the other components of the culture medium were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, Mo., USA) or Merck (Darmstadt, FRG). The organic calcium-channel blocker, verapamil (5-[3,4-dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-*iso*propylvaleronitrile; Sig-

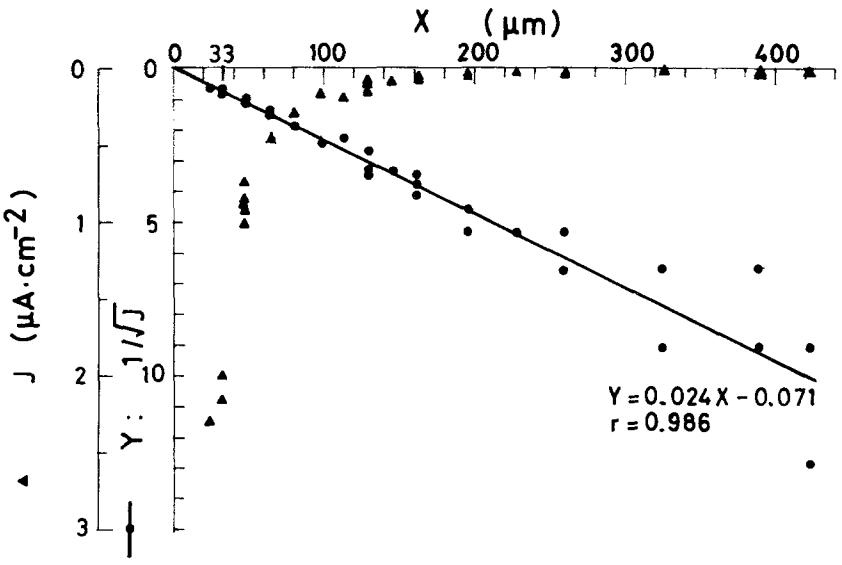

Fig. 2. Relationship between current influx and distance from the apex of *Vaucheria terrestris*. *Ordinates*: Y denotes the reciprocal of the square root of the *J* value (*dots*); the measured *J* values (*triangles*) are also shown in this figure. Each data point represents one measurement from a single apex under continuous BL. *Abscissa*: X denotes the distance between the apex and midpoint of vibration of the probe. The line was drawn by the least-squares method

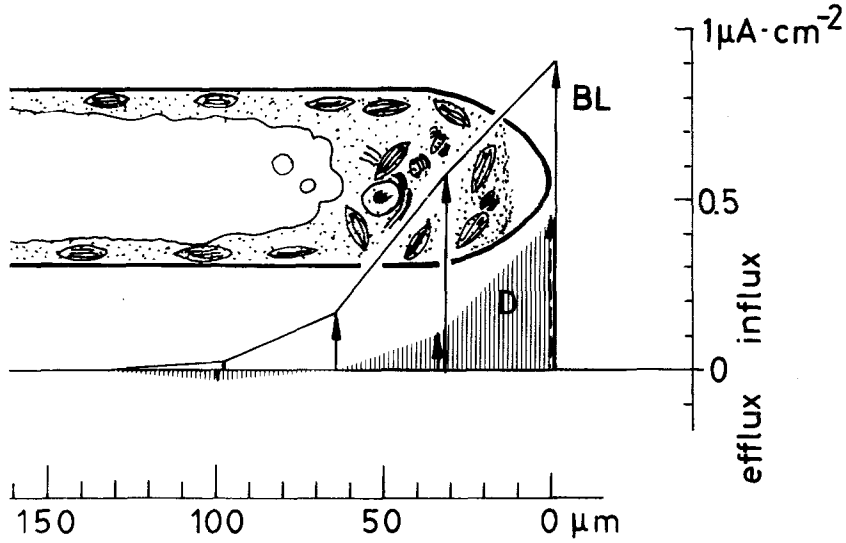

Fig. 3. Extracellular current pattern near the apex *Vaucheria*, with or without BL. The direction of vibration is normal to the cell axis. The cell and the measuring sites are drawn to the same scale. The center of vibration is 57 μm distant from the cell axis. The distance between measuring positions is 33 μm. Current influx in darkness is indicated as shaded area. *D*, darkness; *BL*, blue light

ma; see Kohlhardt et al. 1972) was prepared as 0.1 mM or 0.01 mM stock solution in deionized water and added to the dish with a syringe.

Results

Inward current at the growing apex of Vaucheria terrestris.

The growing apex of *Vaucheria terrestris* is the site of a large inward-directed electric current, as already found in *V. sessilis* (Weisenseel and Kicherer 1981) and other tip-growing cells (Jaffe and Nuccitelli 1977; Weisenseel and Jaffe 1976; Weisenseel et al. 1979). An inward current of a maximum of 3.0 $\mu A \cdot cm^{-2}$ was obtained in front of dark-adapted, rapidly growing *V. terrestris* apices in darkness (i.e., dim green light). This value is of the same order of magnitude as measured in *V. sessilis* (Kicherer 1985). Rapidly growing algae always had large inward currents, and slowly growing ones had small currents.

Figure 2 shows that the magnitude of the inward current is inversely proportional to the square of the distance from the tip. We plotted the reciprocals of the square roots of the *J* values versus the distance from the apex of the alga. The straight line that can be drawn through the points confirms the inverse square-root proportionality. The line extends to the origin of the coordinate axes, indicating that the current sink is directly at the surface. However, this also means that the current density at the surface proper cannot be estimated by extrapolation. Considering the spatial resolution of the measurement, the current density at the surface seems to be rather close to that at

33 μm, i.e., probably about 2.5 $\mu A \cdot cm^{-2}$. Hereafter, unless otherwise stated, J values at the apex are always expressed as those at the 33-μm position.

The electric current was also determined along the cell with the probe vibrating perpendicularly to the cell axis. With this measuring position it can be seen (Fig. 3) that BL irradiation of the apex greatly stimulates the inward current at the apex. Whether the apex is irradiated with BL or kept in darkness, the inward current shows a steep decline towards the basal portion of the cell. In darkness it becomes zero at approx. 65 μm behind the tip, and at 100 μm behind the apex, a very small efflux of approx. 25 $nA \cdot cm^{-2}$ is observed, but there is no appreciable net current at the more basal regions. In BL the inward current extends as far as 130 μm behind the tip, and no outward current was observed at 100 μm. This indicates that in the subapical regions the inward current supersedes the preexisting small outward current. Although no measurable efflux at the basal region can be seen in Fig. 3, we occasionally observed very small (<30 $nA \cdot cm^{-2}$) outward currents at the trunk (100–400 μm below the apex) when the apex was exposed to BL.

The BL-promoted current influx (BLCI). Typical recordings of the BLCI with the probe in front of the apex are shown in Fig. 4. J_D is the J value in darkness and the magnitude of the BLCI is expressed as the net increase of the influx (ΔJ), the difference between the J_{max} and J_D. The J_D values varied widely from apex to apex, depending either on growth rate and size of the cell, but were commonly smaller than 3 $\mu A \cdot cm^{-2}$. As mentioned above, larger J_D values correlate with faster growth rates. To maintain the distance between the probe and the apex constant more easily we chose apices with J_Ds smaller than 0.3 $\mu A \cdot cm^{-2}$, for the following measurements, unless otherwise stated. We commonly observed that every apex had a certain predetermined level of J_{max}, irrespective of its J_D, and hence the larger the J_D, the smaller the BLCI which resulted.

As shown in Fig. 4a, b, with BL pulses shorter than 3 min the inward current increases steeply to a maximum during the first 2–3 min, even after the BL is turned off, and when it reaches its maximum, it starts to decrease again to the pre-light level. A small decrease or transient stagnation is occasionally observed (Fig. 4b) when the BL is terminated. On the other hand, when the BL pulse is longer than 3 min, the maximum current influx continues, although fluctuating in many cases until

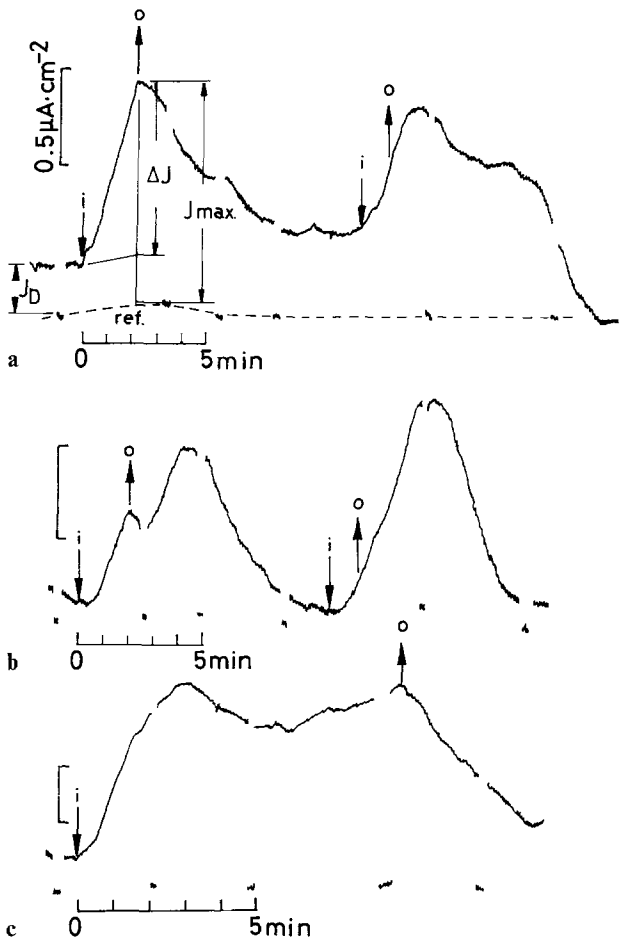

Fig. 4a–c. The BLCI in *Vaucheria terrestris*. Three representative examples of BLCIs redrawn from the chart recorder are shown. Blue light (7 $W \cdot cm^{-2}$) is given at *i* and turned off at *o*. J_D = electrical current in dim green light, i.e., difference between the J value at the measuring position (33 μm) and that at the reference position (ref.); J_{max} = the maximum J value above the reference value; $\Delta J = J_{max} - J_D$. *Vertical bars* represent current influx of 0.5 $\mu A \cdot cm^{-2}$

the end of the BL pulse, and steeply decreases again immediately after the termination of the pulse (Fig. 4c). These time courses of BLCI resemble those of growth response observed earlier (Kataoka 1987). In the present experiments we commonly used pulses shorter than 4 min, because both LGR and phototropic response are brought about by these short pulses.

The BLCI commonly started within 10 s, very frequently within 1 s, although on rare occasions we observed a lag time of 20–30 s (Fig. 4b). These lag phases are obviously shorter than those of the LGR, which were found to be 1–2 min long (Kataoka 1987). When we measured the electric current and the growth rate either simultaneously or separately with individual apices, it became obvious that the BLCI is always accompanied by a

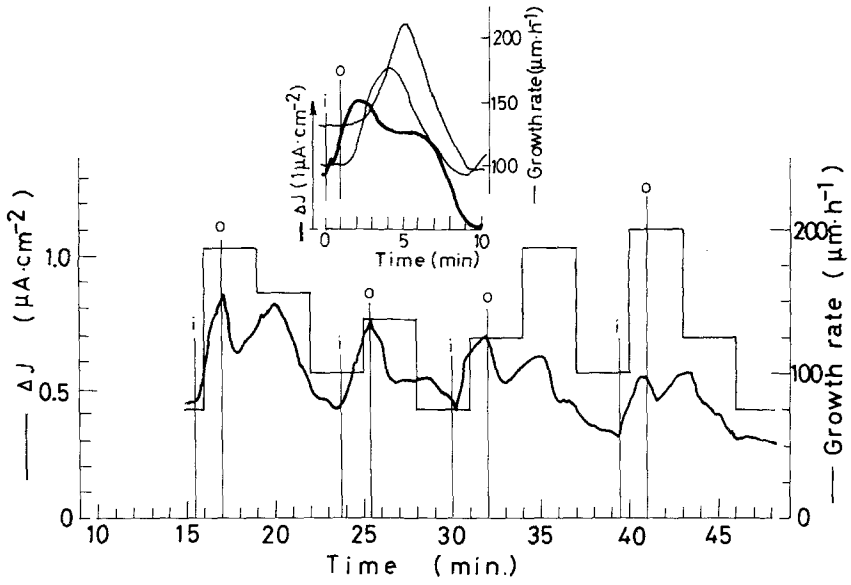

Fig. 5. Timing of BLCI and growth rate in *Vaucheria terrestris*. The data are from a single apex. The BL is turned on at *i* and turned off at *o*. Growth rates determined at intervals of 3 min are represented by steps. *Inset*: Time courses of a BLCI (*heavy line*) and two examples of LGR (*thin lines*) obtained from different cells are superimposed. The growth rates were measured microscopically at every 1 min. A single BL pulse (7 $W \cdot cm^{-2}$ for 1 min) was used to elicit the responses

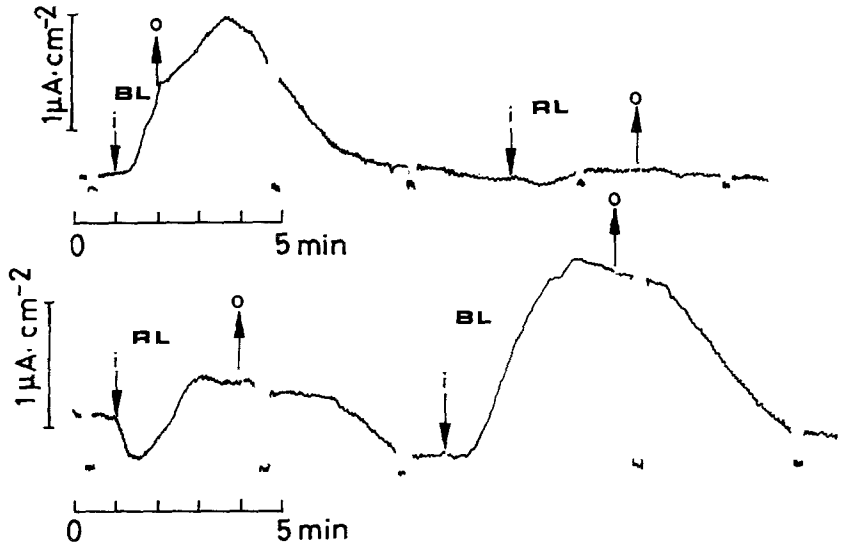

Fig. 6. Effects of RL on the extracellular currents at the apex of *Vaucheria terrestris*. Each trace is a representative example from a single apex. The BLCIs are also shown for comparison.

positive LGR and that the BLCI precedes the LGR (Fig. 5). A maximum BLCI of approx. 5 $\mu A \cdot cm^{-2}$ was observed. This is equivalent to an influx of 50 $pmol \cdot cm^{-2} \cdot s^{-1}$ of monovalent cation, and is of the same order of magnitude as measured in *V. sessilis* apices under continuous white light (Kicherer 1985; Weisenseel and Kicherer 1981).

The effect of the broad-band red light (RL) was completely different from that of BL, because, as shown in Fig. 6, RL caused a transient decrease in inward current at the apex. After this transient decrease, the influx gradually increased under the RL irradiation. The direction of the RL-induced change was the same at the apex and at the basal region, i.e., a stimulation of influx after the initial decrease. This is in contrast to the BL effect, which results in the BLCI at the apex and current efflux, if any, at the trunk of the cell (Fig. 3, Fig. 11). The effect of RL is probably based upon photosynthesis, as suggested by Blatt et al. (1981).

We also observed that repeated BL irradiations with intervals shorter than 10–15 min occasionally led to decreases in BLCI and growth rate. However, we seldom observed such decreases in slowly growing apices. Figure 7 shows a representative example of BLCIs from a single apex with five consecutive BL pulses. It clearly demonstrates that a BL pulse as short as 2 s can cause the BLCI.

As shown in Fig. 8, the magnitude of BLCI (ΔJ) is proportional to the logarithm of pulse-length when the pulse is shorter than approx. 30 s. Extrapolating the lines towards the left, a thresh-

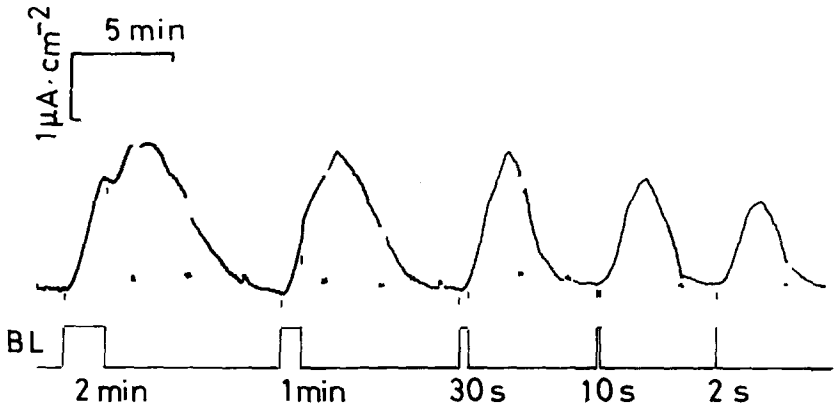

Fig. 7. Successive BLCIs in *Vaucheria terrestris* caused by consecutive BL pulses at a single apex. This is a selected example of almost undiminished BLCIs in APW. The BL irradiations are shown by steps below the trace

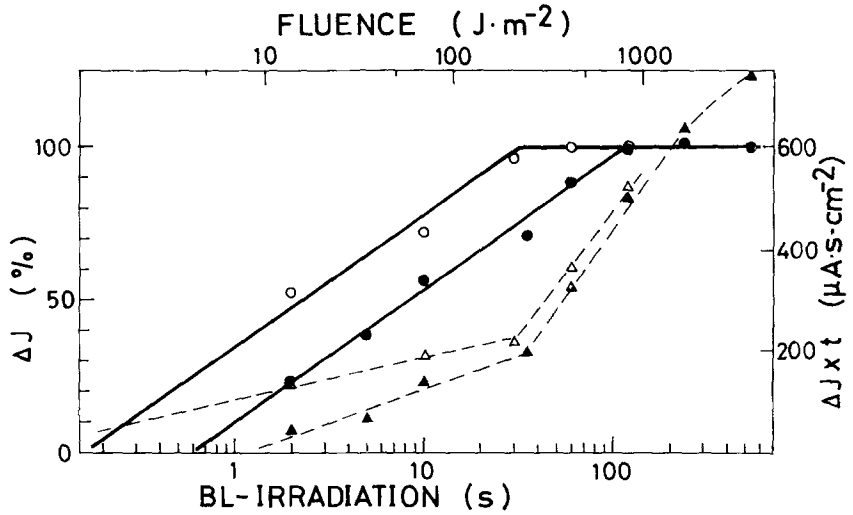

Fig. 8. Fluence-response relationship of in *Vaucheria terrestris*. *Abscissa*: lengths of BL pulses and the corresponding fluence values on a logarithmic scale. *Left ordinate:* normalized ΔJ values from two representative apices (open and closed circles connected by solid lines). *Right ordinate:* the product $\Delta J \cdot t$ of each BLCI from the respective experiments (open and closed triangles, respectively). Each ($\Delta J \cdot t$) value was determined by measurement of the area between the trace of the BLCI and the J_D level on the recorder chart. Data are from Fig. 7 (○, △) and from another representative example of undiminished BLCIs from a single apex (●, ▲)

old fluence of 2–5 $J \cdot m^{-2}$ is obtained. This is smaller than the 10 $J \cdot m^{-2}$ for both LGR and phototropic response previously determined with monochromatic light at 456 nm at pH 7 (Kataoka 1987). Since broad-band BL was used in the present experiments, the exact threshold fluence with monochromatic BL might be even smaller. In Fig. 8 the product of amplitude and time of BLCI ($\Delta J \cdot t$) for the two apices shown is determined by integrating the increments of influx ($J - J_D$) over the duration time. Each curve is composed of two straight lines connected at the point where the ΔJ values reach the saturation level. The threshold fluences for the ($\Delta J \cdot t$) values are similar to those for ΔJ values. This clearly indicates the presence of predetermined saturation level of amplitude (ΔJ) and that the duration of the BLCI increases only after its amplitude has reached saturation. Figure 8 does not, however, demonstrate validity of the reciprocity law because only pulse length was varied in the present study, leaving the fluence rate unchanged.

Effects of pH, Ca^{2+} concentration and verapamil. To investigate the effect of H^+ ions, the alga was transferred to APW with three different pH values, namely, pH 5, 6, and 7, and incubated for 12–24 h in the dark. Then, the BLCI was determined and compared with previous results for the phototropic response and the LGR. Figure 9 shows that in APW with pH 5 only a small BLCI is induced, while at pH 6 the magnitude of the BLCI is equal to (Fig. 9a with 2-min irradiation with BL) or significantly larger (Fig. 9b, 4 min BL) than that at pH 7. In a previous study (Kataoka 1987) photosensitivities of the phototropism and LGR, as expressed by the threshold fluence, were found to be the highest at pH 7, a little lower at pH 6, but only 1/50–1/100 the values of pH 7 at pH 5. Disregarding the difference in the magnitude of BLCI between pH 6 and pH 7 with 4 min irradiation, the pH dependency of the BLCI resembles those of the LGR and phototropism.

After transferring the algae into a Ca-depleted medium (-Ca-APW-7), containing only 0.1 mM

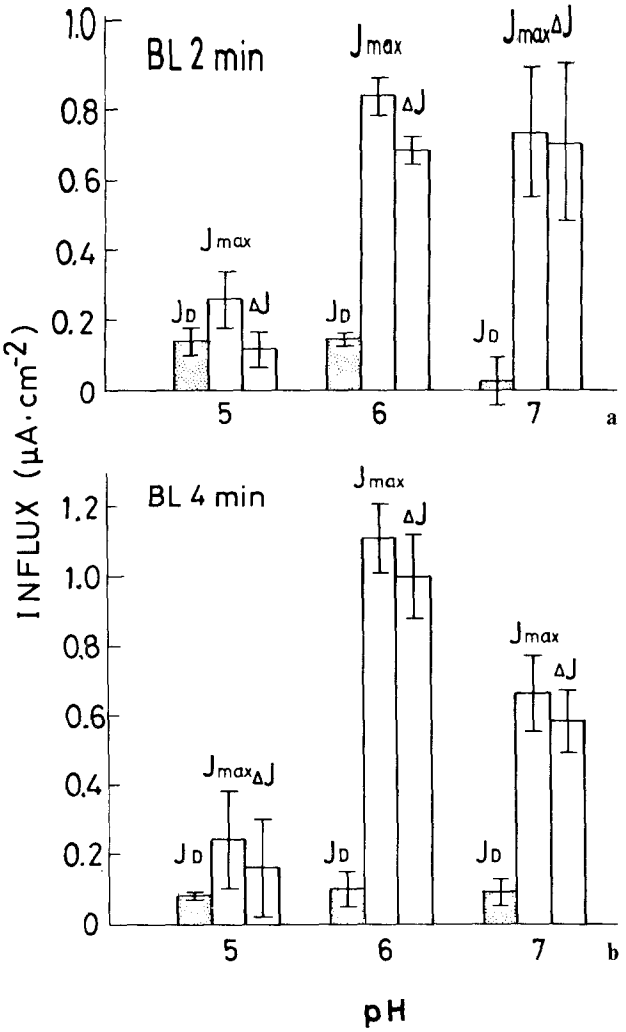

Fig. 9a, b. The pH dependency of BLCI in *Vaucheria terrestris*. **a** With 2-min pulses of BL; **b** with 4-min pulses of BL. Vertical bars = ±SE ($n=6$). Media used are APW-5 (Mes), APW-6 (Mops), and APW-7 (Mops)

KCl, 0.5 mM NaCl and 1 mM Ma-Mops, they grew as fast as in the Ca-containing medium, at least for 5–6 h. However, as shown in Fig. 10, large, but irregularly shaped BLCIs were observed when the alga was irradiated with BL. With the addition of 0.1 mM Ca^{2+}, the BLCI became normal again. We occasionally observed that the addition of 1–10 mM $CaCl_2$ inhibited both BLCI and tip-growth. This indicates that Ca^{2+} ions are not the main component of the BLCI, while approx. 0.1 mM Ca^{2+} is necessary for optimum growth and for the maintenance of normal function of the apical plasmalemma.

We also used an organic Ca-channel blocker, i.e., verapamil, to inhibit the influx of Ca^{2+} at the apex. The addition of 1–20 μM verapamil in the presence of 0.1 mM $CaCl_2$ not only increased the magnitude of BLCI (Fig. 11), but produced undiminished BLCIs to consecutive BL pulses (data not shown). As shown in Fig. 11, the verapamil effect is only seen at the apex, and it is independent of the wavelength of the light, i.e., not only the BLCI is stimulated, but also the RL-induced influx. The addition of 1–2 μM verapamil neither changed the J_D level, nor inhibited tip growth significantly. However, 10 μM verapamil decreased the growth rate from 100–200 $\mu m \cdot h^{-1}$ to about 50 $\mu m \cdot h^{-1}$, and occasionally caused an expansion at the apex. At a concentration higher than 20 μM the drug completely inhibited growth and occasionally caused bursting of the apex.

Discussion

Relationship between the BLCI, LGR and phototropic response. Our results demonstrate:

(1) Blue-light-promoted current influx, LGR and phototropic response are induced by BL, but not at all by RL.

(2) Of the BL-induced growth responses, the BLCI has the smallest threshold value. As determined previously (Kataoka 1987), threshold values for the positive phototropic response and the LGR to monochromatic BL (456 nm) are larger than 10 $J \cdot m^{-2}$ at pH 7. In the present study, a 2-s pulse of broad-band BL (14 $J \cdot m^{-2}$) could eclicit a small BLCI. Taking into consideration the wide range of the actinic light presently used, the real threshold is probably smaller than this value.

(3) The BLCI, the LGR and phototropism are in close spatial and temporal correspondence. All three responses are restricted at the growing apex of the thallus (Kataoka 1980, 1982).

(4) Furthermore, the BLCI seems to precede the LGR (Fig. 5). This inference, however, has to be made with caution, because the BLCI is a directly detectable change in electric current, while the LGR is detected only after calculation based on the increments of length measured at intervals of 1–3 min. Nevertheless, the BLCI seems to be the earliest BL-induced growth-related response of *Vaucheria* so far observed.

(5) In addition, similarity of pH dependency of the three responses (BLCI, LGR and phototropic response) indicates a close relationship. For all these responses neutral to slightly acid pH (pH 6–7) is favorable, and all three are strongly inhibited at pH 5. Furthermore, the result that verapamil increases the BLCI (Fig. 11) even when it strongly inhibits tip-growth (data not shown) indicates that the BLCI is not the result of stimulated growth but rather precedes the LGR. We therefore

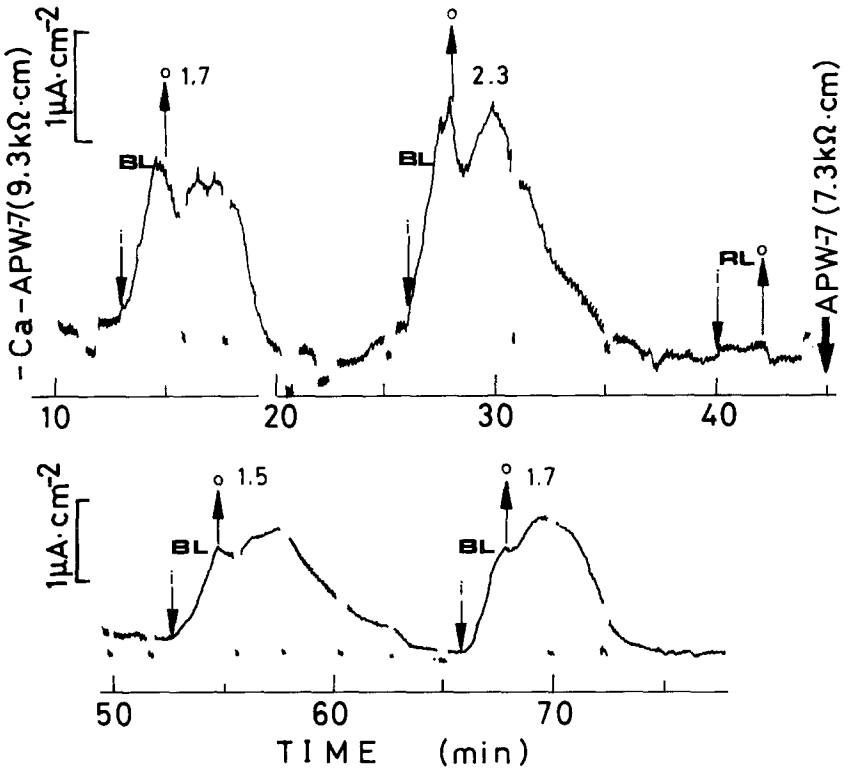

Fig. 10. Enhanced BLCI in *Vaucheria terrestris* in Ca-depleted medium and its depression by the addition of Ca^{2+}. The data are from a single apex. Irradiation with BL and RL are indicated by each pair of arrows, *i*=light on, *o*=light off. The medium, −Ca−APW−7, consisted of 0.1 mM KCl, 0.5 mM NaCl and 0.1 mM Na-Mops adjusted to pH 7. At 45 min the medium was replaced by APW-7. The specific resistivities of the media are also indicated in the figure. The magnitude of the BLCI (ΔJ) is given above each maximum peak

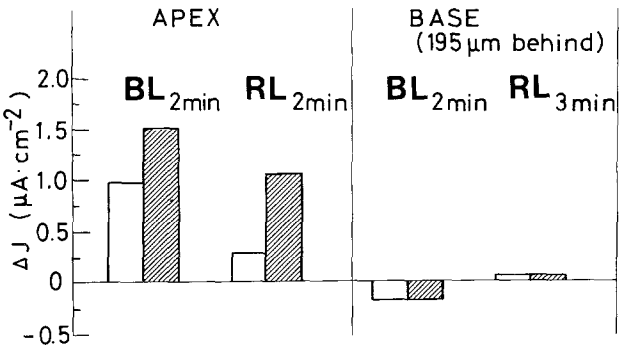

Fig. 11. Effect of 2 μM verapamil on BL- and RL-induced changes in extracellular currents at the apex and 195 μm behind the apex ("BASE") of *Vaucheria terrestris*. Data are from a single cell. *Open columns*: controls (in APW-7); *hatched columns*: currents in the presence of 2 μM verapamil. Each column is the average of light-induced changes of the currents obtained from two or three separate measurements. For measurements at the "BASE" the direction of vibration of the probe was normal to the cell axis (as in Fig. 3). All control data were obtained before the addition of verapamil, and after waiting for approx. 10 min, the remainder of the measurements were made in the presence of the drug. Negative values denote efflux.

propose as a hypothesis that the BLCI is a necessary step of the transduction chain for the LGR and phototropic response of *Vaucheria*.

Nature of the BLCI at the apex of Vaucheria. In the freshwater fungus *Achlya* (Kropf et al. 1984), in roots of barley (Weisenseel et al. 1979) or garden cress (Behrens et al. 1982). Hydrogen ions have been found to be the main component of the inward current. Also, Kicherer (1985) found that under white-light conditions the inward current at the apex of *V. sessilis* was almost of the same magnitude at pH 7 and pH 5, but was strongly inhibited at pH 8, while the tip-growth was completely inhibited at pH 5. She suggested that the influx of H^+ could be the main component of the inward current. In *V. terrestris*, we found that the BLCI was inhibited at pH 5. This does not contradict the earlier findings because the BLCI is not a steady-state inward current but rather a BL-induced increment of the influx. In fact the J_D level was not very dependent on the pH value of the APW (Fig. 9). The fact that the BLCI is larger at pH 6 than at pH 7 (Fig. 9b) indicates an increase of H^+-influx during the BLCI. Since the removal of Ca^{2+} from the medium increased the amplitude of the BLCI, an influx of Ca^{2+} cannot be the main component of the promoted influx of charge.

The BLCI is the largest BL-induced current so far observed in *Vaucheria*. The BL-induced local outward current from the proximal surface of *V. sessilis* found by Blatt et al. (1981), possibly an active proton extrusion, is about 180 $nA \cdot cm^{-2}$ at its maximum; this is at least one order of magni-

tude smaller than the BLCI. We occasionally observed a small increase of efflux at the basal region when the apex was irradiated with BL (Fig. 11). At the BL-irradiated apex, however, we did not observe any efflux as long as the alga was growing. Although these two responses seem to be quite different, they nevertheless might have a common photoreceptor.

The visit of H.K. to the Botanisches Institut I. Universität Karlsruhe was financially supported by the Ministry of Education, Science and Culture, Japan. We thank Professor W. Haupt, Dr. R.M. Kicherer (Institut für Botanik und Pharmazeutische Biologie, Universität Erlangen-Nürnberg, FRG), and Dr. M.R. Blatt (Department of Botany, University of Cambridge, UK) for their valuable comments and advice. The financial support from the Deutsche Forschungsgemeinschaft to M.H.W. is also gratefully acknowledged.

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Received 26 June; accepted 13 October 1987