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Blue-Light-induced Cortical Fiber Reticulation Concomitant with Chloroplast Aggregation in the Alga *Vaucheria sessilis**

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Abstract. Point illumination with low-intensity blue light induces the chloroplasts and other organelles, which normally stream in the cytoplasm of *Vaucheria sessilis* (Vauch.) D.C. (Xanthophyceae), to aggregate in the illuminated region of the cell. Aggregation is passive and results from the "trapping" of the organelles as they stream into the blue light. Prior to illumination, longitudinal fibers along which the organelles appear to move, can readily be seen through a light microscope fitted with differential interference contrast optics. Upon actinic irradiation, these fibers appear to become destabilized, branching and forming a cortical fiber reticulum in the light. The reticulation process always precedes chloroplast aggregation. Aggregation itself occurs after a lag period which is inversely related to fluence rate. The lag period at high fluence rates (>400 mW m^{-2}) may be as short as 20 s. Studies of wavelength dependence show that wavelengths near 480 nm are maximally effective while those longer than 530 nm are inactive.

Key words: Blue-light responses – Chloroplasts (movement) – Cortical fiber reticulation – Light (blue) – *Vaucheria*.

Introduction

The chloroplasts of many higher plants and algae undergo subcellular redistribution in response to directional illumination. This phenomenon includes the promotion or inhibition of chloroplast movement from one place in the cell to another by light as well as light-induced alterations of chloroplast orientation and shape (Haupt and Schönbohm, 1970; Ohiwa, 1977). These movements depend both upon the spatial distribution of incident light within the cell as well as upon its intensity. They enable the plant, on the one hand, to realize its maximal photosynthetic potential under less than optimal conditions of illumination and, on the other, to protect the chloroplast from high and presumably damaging light intensities (Lechowski, 1974; Zurzycki, 1955).

Early theories held that the chloroplast itself was both light-sensitive and capable of self-propulsion through the protoplasm (see Senn, 1908). However, evidence accumulated in the course of the past quarter century has demonstrated that, in general, the photoreceptor is not associated with the chloroplast. The photosynthetic pigments mediate neither the positive nor the negative photoresponses. The action spectra in most instances indicate, rather, that the photosensory pigments are flavoproteins (see review by Haupt and Schönbohm, 1970), and experiments with quenchers support this hypothesis (Schönbohm, 1969; Seitz, 1970). (*Mougeotia* and *Mesotaenium* are notable exceptions in which the positive photoresponses are mediated by phytochrome.) Furthermore, experiments employing irradiations with microbeams or with polarized light (or both) have demonstrated that the primary photoreceptor is not located in the chloroplast, but is stationary within the cortical region of the cytoplasm (Haupt, 1970; Mayer, 1964; Fischer-Arnold, 1963).

In all likelihood, the sensory transducing mechanism responsible for the light-induced orientation movements is also separate from the chloroplast (cf. review by Haupt, 1970; Fischer-Arnold, 1963). Nevertheless, mechanistically, the link between photon absorption and chloroplast movement and orientation remains largely a mystery. Indirect evidence indicates that the light-induced orientations and movements of the chloroplasts of *Mougeotia* (Wagner et al., 1972; Schönbohm, 1973a, 1975) and *Funaria* (Schönbohm, 1975) are dependent upon an actomyosin system. Thus, at least in plants exhibiting directed chlo-

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roplast motion in response to light, chloroplast orientation movements could reflect a regulatory coupling of light to the molecular machinery of cellular and subcellular motility (Goldman et al., 1976). Indeed, Schönbohm (1975) has observed that the number and arrangement of cytoplasmic fibers associated with the chloroplasts of *Funaria* and *Mougeotia* are altered coincident with the initiation of the photoresponse. The results indicate that light could have its effect by acting in some way on a microfilament system.

In contrast, the chloroplasts of many higher plants, such as *Elodea* and *Vallisneria* (see review by Zurzycki, 1962), and some algae, including *Vaucheria* (Fischer-Arnold, 1963), move with the streaming of the cytoplasm even in the absence of light. Light, in these instances, alters or inhibits movement of organelles to varying degrees.

In this first of two papers, we present evidence that the inhibition of chloroplast movement by light in the alga *Vaucheria sessilis* is preceded by, and possibly coupled to, the localized formation of a cortical fiber reticulum in the light.

Material and Methods

Algal Culture

Vaucheria sessilis (Vauch.) D.C. (Xanthophyceae), originally obtained from the University of Texas (Austin) Culture Collection, was grown either on 1% bactoagar or in liquid medium. Axenic cultures, isolated as described by Åberg and Fries (1976), were maintained on 1% bactoagar plates. The growth medium, designated 0.75mBl, consisted of a $^3/_4$-strength modified Bold's medium (see Starr, 1960), with the addition of micronutrients as described by Waris (1953) for his MXS medium and 0.2 mM tris-(hydroxymethyl)aminomethane (Tris) (from the Sigma Chemical Co., St. Louis, Mo., USA). The pH was adjusted to 7.7 with 1M KOH or HCl. With the isolation of axenic cultures of the algae, the further addition of 100 mg/ml biotin, 2 µg/ml vitamin B_{12} and 50 µg/ml thiamine-HCl (all from Sigma Chemical Co.), designated BBT, was made following sterilization of the agar and liquid media. BBT was sterilized by passage through a Millipore filter (Millipore Co., Bedford, Mass., USA) and stored frozen in 4-ml aliquots. Stock cultures growing on agar were transferred monthly, and some of the algal filaments were transferred at the same time to liquid culture for experimentation during the subsequent month. All cultures were kept on a 16-h day:8-h night cycle in a Percival growth chamber. Lighting (10 W m^{-2}) was provided by two 40-W Sylvania fluorescent lamps and the day temperature was held at 21° C (±0.5° C) during the light period and at 17° C during the dark period.

Light Microscopy

Whole algal filaments were examined with Zeiss Nomarski Differential Interference Contrast (D.I.C.) optics on a Zeiss Universal Microscope. Planapo 16×, 40× and 63× (the latter with oil immersion) objectives were used with 10× oculars for observation and photography.

Light Sources, Measurement

Non-actinic background light, provided by the microscope illumination system and a Zeiss HB0200 mercury arc lamp, was first filtered with a 560-nm cut-off filter (Corning CS3-66) and an additional heat filter (Corning CS1-69). Actinic irradiation through the microscope condenser was achieved by combining this beam with the condensed beam from a second Zeiss HB0200 mercury arc lamp with a 45° half-silvered mirror (Balzer's TF-MT-45) positioned directly beneath the microscope condenser. Light from this second lamp was also filtered to remove infrared wavelengths (Corning CS1-69). Thereafter, the beam intensity could be reduced with neutral density filters, and various wavelengths selected by a suitable choice of interference and/or cut-off filters. The diameter of the condensed beam in the focal plane of the microscope was ca. 30 µm.

The intensity of the actinic beam transmitted by the selected interference filters was measured with a 5-mm diameter silicon solar cell (Edmund Scientific Co., Barrington, N.J., USA), which was placed on a microscope slide and positioned in the focal plane of the microscope. The silicon cell output was monitored on a Keithley 150B microvoltmeter (Keithley Instruments, Cleveland, O., USA). This system was calibrated against a quantum sensor (Model LI-190S, LI-COR, Lincoln, Neb., USA). Once positioned on the microscope stage, the cell was irradiated and the actinic beam was spread until the voltage output reached a maximum; the maximum was obtained when the entire surface of the solar cell was illuminated. The maximum voltage was recorded and the beam intensity then back-calculated to the original beam diameter.

In order to determine the wavelength sensitivity of the reticulation process, interference filters (B-40 series; Balzer's Liechtenstein) were combined with appropriate neutral density filters to give a constant subsaturating fluence rate for the actinic beam of 300 mW m^{-2}, irrespective of the wavelength selected. The half band-widths for the interference filters used are shown in Table 1. The wavelength sensitivity for the photoresponse was measured as a function of the time necessary to initiate reticulum formation (see below) following the onset of actinic irradiation. Any bias from the observer was eliminated by the fact that the actinic beam was entirely masked by the background illumination. Additionally, the filter combination for each wavelength were placed, at random, in the path of the actinic beam by another person, so that the observer was unaware of the test wavelength. A similar procedure was followed in the measurement of the intensity-versus-time relationship for 478-nm light, but in this case neutral density filters only were removed from or replaced at random in the actinic beam path by another person.

Experimental Protocol

To insure further uniformity in measurement, all algal filaments to be used on a given day were removed from the growth chamber

Table 1. Half band-widths for the Balzer's B-40 series interference filters employed in the various actinic irradiations. Wavelengths and half band widths are expressed in nm

Wavelength	Half band width	Wavelength	Half band width
385	11	467	10
395	16	478	8
401	11	486	11
415	10	496	11
432	12	503	9
452	14	512	9
462	8	534	9

during the preceding dark period and kept in the dark for observations and/or measurements between the 2nd and 5th hour of what would have been their light period. All operations were performed under dim green safelight (emission maximum 530 nm, half bandwidth 10 nm) and the ambient temperature remained at $22.5 \pm 0.5°$ C at all times. For direct microscope observation and measurement, algal cells in 0.75 mBl ± BBT were placed on glass slides and sandwiched between the slide and coverglass. Preparation of the algae in 0.75 mBl ± BBT for measurement of the kinetics of aggregation and the operation of the two-wavelength recording microphotometer utilizing optical fibers is described in Blatt and Briggs (1977). (Minor changes in electronics will be described elsewhere).

Photography

Photographs were taken with a Zeiss 35-mm camera with automatic shutter and recorded on Kodak Tri-X Pan film (Eastman Kodak, Rochester, N.Y., USA). Cinematographic records of aggregation were taken with a Sage time-lapse 16 mm camera system (Series 500 Cinephotomicrographic Apparatus; Sage Instruments, White Plains, N.Y., USA) at two frames per second on Kodak Tri-X or 4-X 16 mm reversal film.

Results

Vaucheria sessilis is one of a small group of filamentous algae common to brackish waters. Filaments of *V. sessilis* are typically several centimeters in length with diameters varying from 50 to 150 μm. *Vaucheria* filaments are coencytic, and form crosswalls only when developing reproductive structures or sealing off an injury. The algal filament can thus be envisaged as a hollow cylinder of cytoplasm, one or a few micrometers thick, containing numerous small (3–5 μm in diameter), plate-like chloroplasts and prominent oil droplets, surrounding a single, large central vacuole, and bounded on the outside by the cell wall. The chloroplasts of *Vaucheria* are motile and stream, accompanied by small vesicles and mitochondria, in a multistriate fashion (along several channels; see review by Kamiya, 1962) along the long axis of the cell. Streaming is slow, averaging, in a healthy, dark-adapted filament, between 17 and 20 μm min^{-1} (Blatt and Briggs, 1978).

Our preliminary observations showed the presence of cytoplasmic fibers, ca. 0.5 μm in diameter, longitudinally oriented in the cortical region of the cytoplasm (Fig. 1). Using Nomarski D.I.C. optics we found that these fibers are closely associated with chloroplasts, mitochondria and vesicles, and appear to guide the streaming of these organelles. Several centimeters back from the growing tips of the algae the number of cortical fibers is often reduced (see Fig. 5). Under these circumstances, it is clear that streaming only occurs along the fibers. Curiously, organelles will move in both directions along a single fiber, and may even pass one another. Collisions between chloroplasts are common, after which both organelles will continue their movement. In some instances, chloroplasts may also travel in a helical pattern around a single fiber. Thus, an organelle will shift in and out of the plane of the fiber, as it proceeds along the fiber, as well as moving from one side of it to the other and back. Ott and Brown (1974) have reported similar fibers in filaments of *V. litorea,* but have not reported any associated directional chloroplast movements. They describe the chloroplasts as being in random motion, a behavior that is reminiscent of the negative photoresponse of chloroplasts (Fischer-Arnold, 1963; Haupt and Schönfeld, 1962),

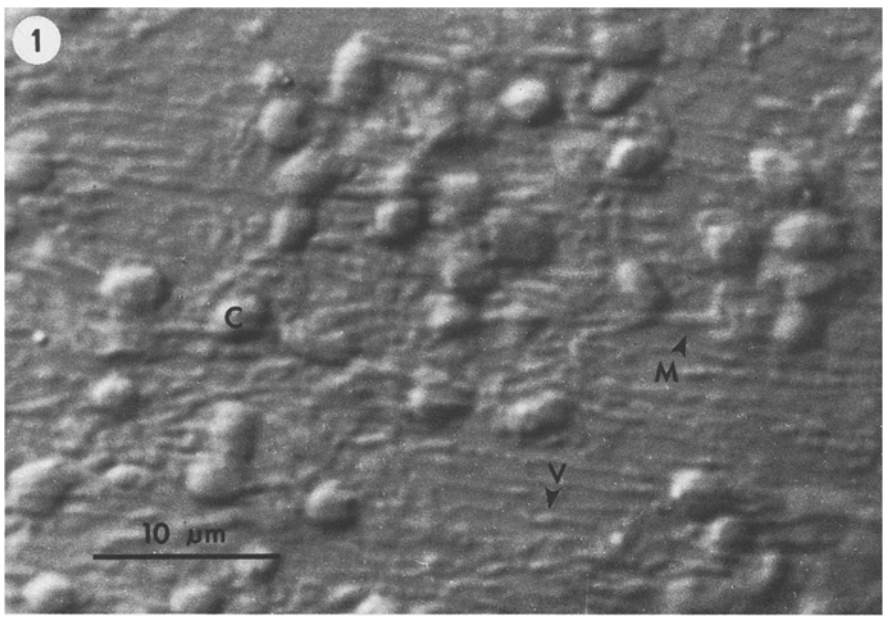
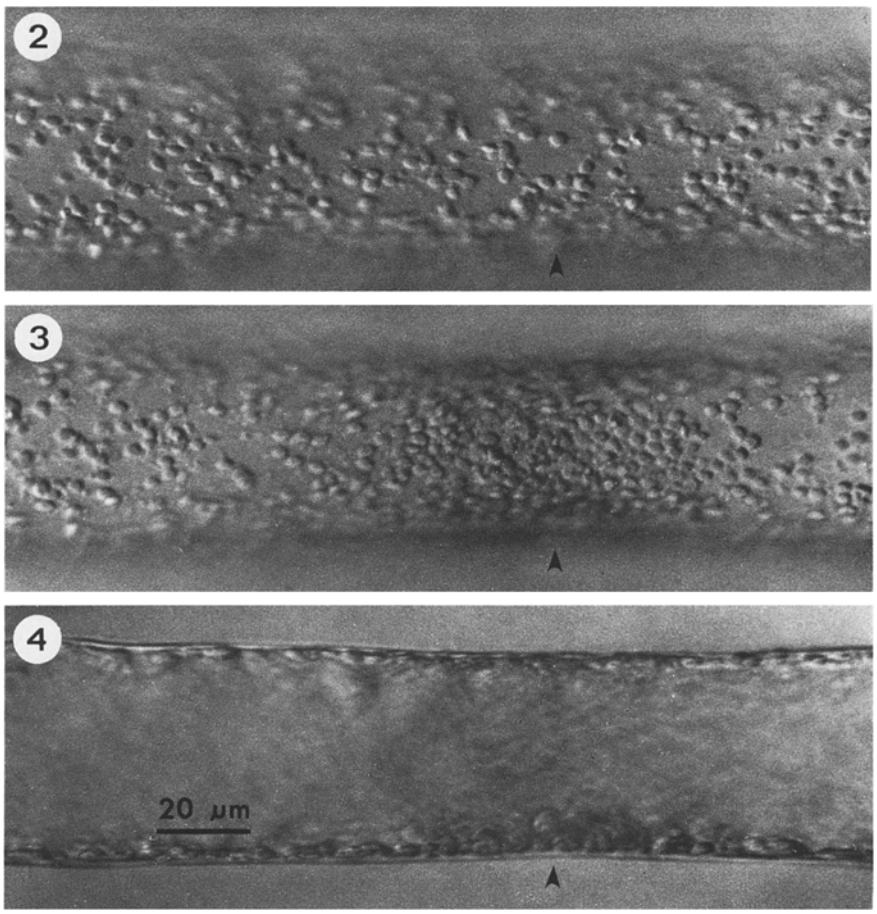


Fig. 1. *Vaucheria sessilis*. In grazing optical section and with Nomarski D.I.C. optics, numerous fibers can be seen. They are roughly 0.5 μm in diameter and longitudinally oriented within the cell (the cell axis in this micrograph is horizontal). Chloroplasts (*C*), 3 μm in diameter, smaller vesicles (*V*), and what are probably mitochondria (*M*) can also be seen. × 2,400



Figs. 2–4. *Vaucheria sessilis*. Choroplast aggregation in response to low-intensity blue light (478 nm, Balzer's B-40, half band-width 8 nm) as seen with Nomarski D.I.C. optics. The algal filament pictured in these figures was illuminated by a single, 50-μm diameter optical fiber inserted between the microscope slide and the coverglass, and positioned against the algal cell (centered at the arrow). The optical fiber was withdrawn from view for these micrographs, since diffraction of the light from the microscope field illuminator by the optical fiber distorts the view of the adjacent algal cell. × 600

Fig. 2. A grazing optical section of the algal filament prior to actinic illumination. Note the uniform distribution of the chloroplasts

Fig. 3. The same portion of the algal filament in grazing optical section after 5 min of actinic irradiation. Aggregation extends throughout a narrow region of the cell, but is most pronounced in those regions of the alga which face directly onto the optical fiber (see Fig. 4)

Fig. 4. As Fig. 3, but filament in longitudinal cross section

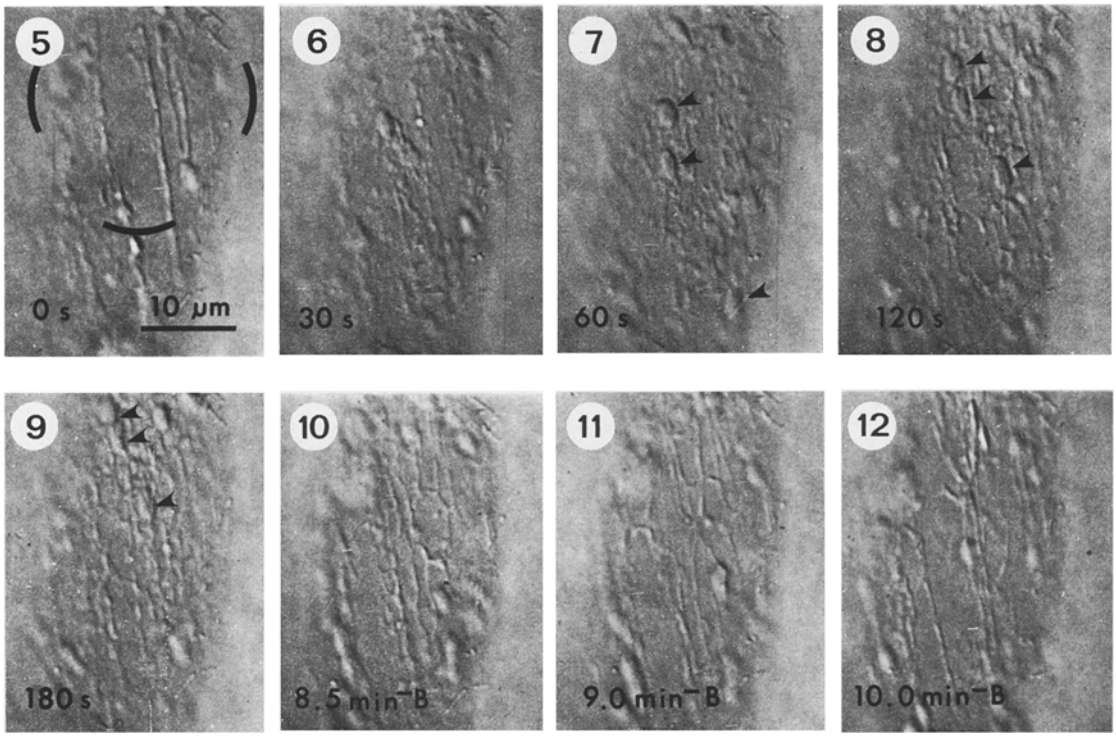
and was probably induced by the high intensity white light used to illuminate the microscope field.

In *V. sessilis,* the fibers themselves are not entirely stationary and occasionally wobble or bifurcate, indicating that they, in fact, represent cables composed of smaller fibers. Nor are they necessarily aligned with the long axis of the cell. Scanning along an algal filament, one sees that the cortical fiber array may abruptly assume a helical pattern around the cell and continue spiralling for several hundreds of micrometers. The fiber pattern, however, whether longitudinal or helical, is stable, at least for 3 h.

If a small region of a dark-adapted filament of *V. sessilis* is illuminated unilaterally with actinic blue light at a fluence rate that saturates the positive photoresponse (500 mW m^{-2}; see Fischer-Arnold, 1963), after a 20-s lag, any chloroplasts, vesicles and mitochondria which stream into the illuminated region from either direction stop once in it. This "trapping" of organelles in the light, which constitutes the positive photoresponse, is rapid and reversible. Within 5–10 min an aggregate of organelles forms on the side of the algal filament facing the light, and to a lesser extent on the side away from the light, and frequently bulges as much as 10–20 μm into the vacuolar region of the cell (Figs. 2–4). If the actinic beam is removed, normal streaming is resumed and the aggregate disperses within 15–30 min.

The movement of the organelles within the aggregate does not cease entirely, but it is limited and non-directional. Saltatory movements of individual chloroplasts can be observed to occur in all directions within the aggregate, and organelles trapped at the perimeter of the aggregate do occasionally escape. Nevertheless, as organelles streaming into the actinic beam become trapped, and as active streaming continues in both directions along the longitudinal axis of the cell outside the perimeter of the actinic beam, prolonged actinic irradiation will deplete these adjacent regions of the algal filament of chloroplasts and other organelles. Similar observations have been made by Senn (1908) and Fischer-Arnold (1963).

Low-fluence-rate blue light also has a profound influence on the organization of the cortical fibers and their spacial arrangement within the cell. The frames in Figs. 5–9, taken from a time-lapse film of aggregation, show the typical sequence of events during the first 3 min of low-fluence 478-nm irradia-



Figs. 5–12. *Vaucheria sessilis* cortical fiber reticulation induced by low intensity (250 mW m^{-2}) 478 nm light (Balzer's B-40, half band width 8 nm). Grazing optical sections of a single algal filament permit us to follow the course of events during the first minutes of blue-light irradiation, and following the removal of the actinic beam, as recorded by time-lapse cinematography. The cell axis runs longitudinally in each of the film frames, and the area exposed to the actinic beam is encircled in the first frame. The time elapsed (in seconds) following the onset of actinic irradiation is indicated in the first five frames, and the minutes elapsed following the removal of the actinic beam are given in the last three frames. ×830

Fig. 5. (t=0 s). The onset of actinic irradiation. A few, very distinctive, longitudinally-oriented, cortical fibers are evident

Fig. 6. (+30 s blue light). Initial reticulation is evident in the branching of the fibers. Streaming continues unaltered in the actinic beam at this time. Note the finer appearance of the fibers in comparison with Fig. 5

Fig. 7. (+60 s blue light). The onset of aggregation. Streaming organelles become trapped in the light. Arrows mark the positions of three chloroplasts in this and the subsequent two figures. A fine fiber reticulum is just visible here, as well as in Figs. 8 and 9

Fig. 8. (+120 s blue light). Note that with the onset of aggregation, the three chloroplasts remain essentially stationary

Fig. 9. (+180 s blue light) same as in Fig. 8

Fig. 10. (+8.5 min after the actinic beam was removed). When the blue light is turned off, the cortical fiber reticulum opens, and, over the course of 10–15 min, the fiber pattern assumes its original appearance. Several large fiber loops are clearly visible here, the fibers are fewer in number, and appear larger and more distinct. A number of chloroplasts may be seen below focus to the left

Fig. 11. (+9.0 min after the blue light was removed) same as in Fig. 10

Fig. 12. (±10.0 min after the blue light was removed). Note that the fiber pattern begins to resemble that of Fig. 5

tion (250 mW m^{-2}). Nomarski D.I.C. optics were employed, as before, to section the algal filament optically and expose the upper cytoplasmic surface. The long axis of the cell is oriented vertical to the page, and the area exposed to the actinic beam is encircled in the first frame. (The fluence rate of blue light used to excite the positive photoresponse was too low to be seen against the background illumination necessary for viewing and filming). The time elapsed in seconds, following the onset of actinic irradiation (at $t=0$) is indicated in each frame.

For this film sequence we intentionally chose a region of algal filament with relatively few chloroplasts so that the view of the cortical fibers would not be obscured by a background of numerous organelles. Thus, an extensive aggregate is not in evidence in these frames. Small organelles and a few chloroplasts (Figs. 7–9, pointers) were trapped in the

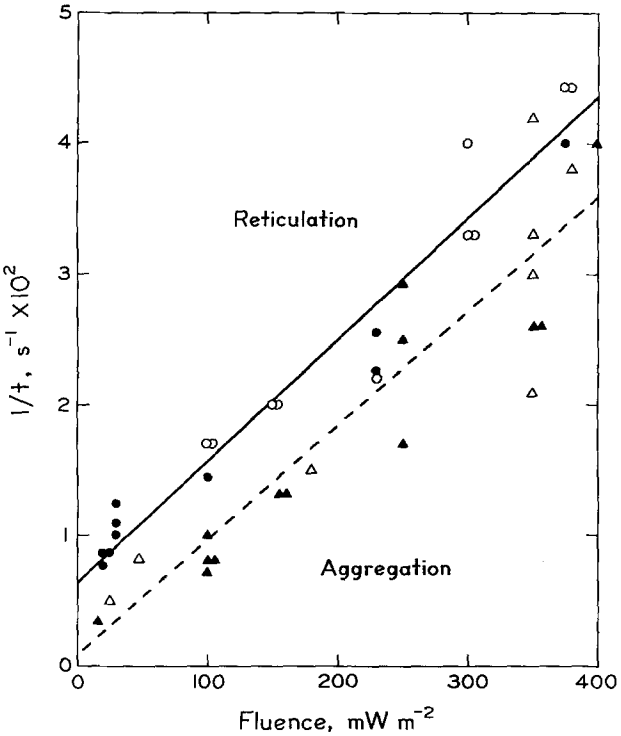


Fig. 13. Intensity vs. inverse time dependence for initial cortical fiber reticulation (circles and solid line), and for the latency period duration prior to chloroplast aggregation (triangles and dashed line) in *Vaucheria sessilis*. Measurements were taken from young algal filaments (within 3 cm of a growing tip; ○ and △) and from mature filaments (at least 5 cm from any growing tip; ● and ▲). A 478-nm actinic beam (Balzer's B-40 interference filter; HBW 8 nm) was used. Fiber reticulation was determined visually with the aid of Nomarski D.I.C. optics, as described in the Methods section. The time points for initial chloroplast aggregation following the onset of actinic irradiation were taken directly from transmission recordings similar to Fig. 14. Reticulation: slope, $9.2 \times 10^{-5} m^2/mW$; linear regression coefficient, 0.95; aggregation: slope, $8.6 \times 10^{-5} m^2/mW$; linear regression coefficient, 0.84. The designations "young" and "mature" filaments are somewhat arbitrary, but reflect the local distribution of cytoplasm and organelles as well as the local capacity for vegetative growth (Ott and Brown, 1974; Aberg and Fries, 1976)

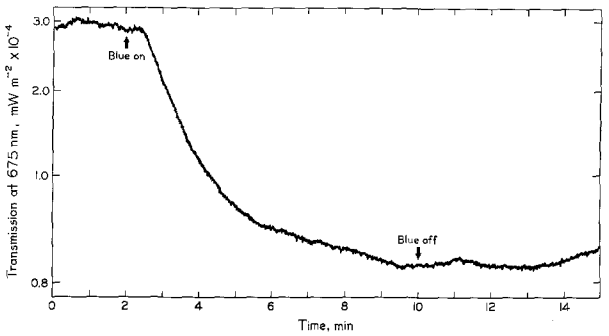


Fig. 14. A sample transmission recording of chloroplast aggregation in *Vaucheria sessilis*, from a two-wavelength recording microphotometer (Blatt and Briggs, 1977). Collimated and filtered actinic and measuring light beams from two sources are combined with a cold mirror (Balzer's) and focussed on one end of the optical fiber used for point illumination of the alga. Light transmitted through the alga is collected by a second optical fiber that conducts it to a phototransistor. A filter placed between the collecting fiber and phototransistor removes the transmitted actinic blue light and passes the 675-nm light of the measuring beam. The signal from the phototransistor is then amplified and displayed on a chart recorder. Fluence rates: 675 nm, $9.6 \times 10^3 mW\ m^{-2}$; 478 nm, 400 mW m^{-2}. Chloroplast aggregation, characterized by a rapid drop in transmitted red light, begins 25 s after the onset of 478 nm irradiation. When the blue light is removed, the aggregate begins to disperse and 675 nm transmission slowly climbs to its original value (not shown)

lighted region during the 2nd minute of the blue irradiation, and we can estimate that aggregation, in this instance, should have begun 40–60 s following the onset of actinic irradiation (see Fig. 13).

As is evident from the frames in Figs. 5–9, blue light induces a marked shift in the arrangement of the cortical fibers visible with Nomarski D.I.C. optics. Whereas prior to illumination the fibers are aligned essentially parallel to the long axis of the cell, under continuous blue light the fibers bifurcate and anastamose, increasing in number as they decrease in diameter. The resulting fiber reticulum is visible in Figs. 8 and 9. The first signs of this behavior can be seen 30 s following the onset of actinic illumination (Fig. 6), or at least 10 s prior to the first visible signs of aggregation (Figs. 7–9), eventually extending throughout, but largely limited to, the illuminated region of the alga. Both during the reticulation process and within the reticulum itself, fibers will appear and disappear from view, and will open and branch in a continuous fluid motion, indicating that the fibers are unstable in the light. If the actinic beam is removed the process is reversed, the reticulum opens (Figs. 10, 11), and after 10–15 min (Fig. 12) the fiber pattern begins to assume its original appearance.

This complex light response, involving reticulation followed by organelle aggregation, and its subsequent relaxation in the dark, can be obtained repeatedly in a given region of the filament. Figure 13 shows the relationship between actinic fluence rate and the reciprocal of the time at which the first sign of reticulation and chloroplast aggregation can be observed. Reticulation invariably preceeds aggregation irrespective of actinic light fluence rate. Additionally, the two processes share a common dose dependence at this wavelength. Time points for fiber reticulation were measured as described above, to determine the first observable signs of fiber bifurcation. We followed the kinetics of aggregation employing a two-wavelength recording microphotometer described elsewhere (Blatt and Briggs, 1977). A sample transmission recording is presented in Fig. 14. The time point for the start of aggregate formation was taken directly from the transmission recordings. Aggregate

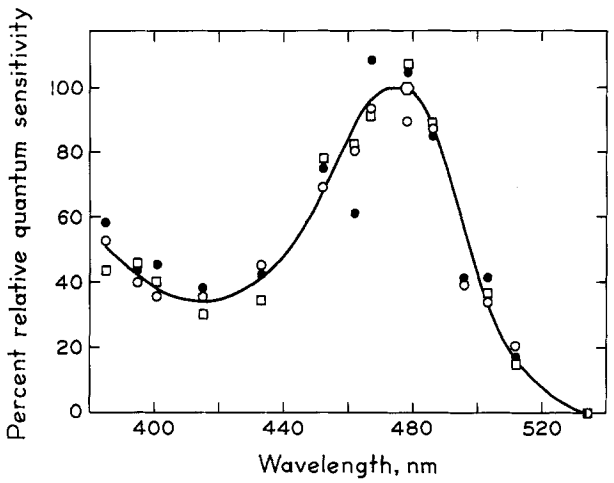


Fig. 15. The wavelength dependence of the quantum requirement necessary to initiate cortical fiber reticulation in *Vaucheria sessilis*, expressed as the relative quantum sensitivity, with the mean of the measured values at 478 nm (41 s) taken as 100% (○). Measurements of the duration of the latency period between the onset of actinic irradiation and the first visible signs of fiber reticulation, as described in the Material and Methods section, were taken from three separate algal filaments, 2 cm (△), 5 cm (○), and 7 cm (□) from the nearest growing tips, irradiated with a standard actinic beam intensity (300 mW m^{-2}). The quantum flux density at each wavelength was determined later with the assumption that all of the energy transmitted by the various interference filters corresponded to their respective wavelength maxima. The half band widths (in nm) for each filter are shown in Table 1. No response resulted from irradiation with wavelengths above 560 nm (Corning) CS3-66; not shown)

formation is, of course, dependent both upon the chloroplast density and upon the rate of chloroplast streaming within the cell. Initiation of the photoresponse, however, is independent of these factors (see Fischer-Arnold, 1963), and does not vary substantially from cell to cell or as the algal filaments age.

We have examined the spectral sensitivity of the reticulation response and our results indicate that, like aggregation, reticulation is maximally sensitive to blue light around 460–480 nm and insensitive to wavelengths of light longer than 520 nm (Fig. 15). It has not been possible to test this photoresponse for reciprocity, and thus Fig. 15 does not represent a true action spectrum. Nevertheless, the light sensitivity of reticulation shown here is similar to the action spectrum for aggregation (Fischer-Arnold, 1963).

Discussion

Fischer-Arnold (1963) found that the photoreceptor for chloroplast aggregation in *Vaucheria* was stationary within the cytoplasm. By point irradiation of algal filaments with 12-μm beams of actinic light she was able to demonstrate that aggregation occurred whether or not chloroplasts were present within the beam perimeter during the latency period. Furthermore, by tracking individual organelles with the actinic beam she found that irradiation of a fixed area of the cell throughout the latency period was a necessary and sufficient condition for the initiation of the photoresponse in that region.

Although Fischer-Arnold's observations do not permit localization of the effector for aggregation, it is clear that this effector system, too, cannot be freely mobile in the cytoplasm, at least during light stimulation. Thus, the blue light-sensitive photoreceptor-effector system could be located at the plasma membrane, the tonoplast, in association with the cortical fibers, or any combination thereof. Our observations indicate that blue light induces the local formation of a reticulum of cortical fibers, which in the dark-adapted alga are longitudinally oriented and are associated with the streaming of organelles. Phenomenologically, reticulation precedes aggregation, and both photoresponses are reversible within roughly the same time period. Preliminary kinetic and spectral data also indicate that these photoresponses are closely related.

Cytoplasmic fibers and changes in intracellular fiber patterns in conjunction with light-induced chloroplast orientation movements are not unique to *Vaucheria*. Schönbohm (1975) has observed cytoplasmic fibers with differential interference contrast microscopy; these fibers may represent large aggregates of microfilaments, stretching between the chloroplast and cortical cytoplasm in *Mougeotia*, and extending between chloroplasts in the cytoplasm of *Funaria*. These fibers appear to guide the streaming of small particles and may themselves vibrate, extend and retract. The number of fibers visible is dependent upon the recent history of illumination. Schoenbohm found that the number of fibers increased at low intensities and at wavelengths which induced positive chloroplast orientation movements, and their appearance coincided with the first sign of movement. In *Mougeotia*, this increase was also dependent upon the orientation of polarized red light, and could be reversed by far-red irradiation, paralleling the orientation behavior of the chloroplast. It is noteworthy that the photoresponse in *Mougeotia* involves directed movement of the chloroplast in response to unilateral illumination. In this instance, the mobilization and coupling of the system generating motive force to the chloroplast could be expected to precede chloroplast rotation. This proposal finds support in inhibitor studies (Wagner et al., 1972; Schönbohm, 1975), which indicated involvement of an actomyosin-mediated shear force, and bundles of actin-like filaments have been observed between the chloroplast and cortical cytoplasm in *Mougeotia* (Wagner and Klein, 1978).

In contrast, aggregation in *Vaucheria* reflects the loss of normal organellar streaming behavior. Yet here, too , as in *Mougeotia,* light which induces the positive photoresponse also has a profound effect on the cortical fiber organization. Fischer-Arnold (1963) interpreted the cessation of streaming in *Vaucheria* in response to actinic irradiation as indicative of a localized gelation of the cytoplasm. Historically, the rate at which organelles could be centrifuged in vivo has been used as a measure of cytoplasmic viscosity (Zurzycki, 1962). Indeed, when plant cells exhibiting chloroplast orientation movements are pretreated with light which stimulates the positive photoresponse, the sensitivity of the chloroplasts to applied centrifugal forces will in many cases decrease (cf. Virgin, 1952, and his review, 1964). Nevertheless, pretreatment of *Mougeotia,* in which chloroplast orientation movements clearly are not dependent on cytoplasmic gelation, with red light will also decrease the chloroplast sensitivity to centrifugation (Schönbohm, 1973b).

We suspect that cortical fiber reticulation in *Vaucheria* reflects a more specific and subtle intracellular influence of light than cytoplasmic gelation implies. We have noted that both during the reticulation process and within the reticulum itself the cortical fibers appear unstable, branching, anastamosing, and undulating continuously. Since the cortical fibers in *Vaucheria* are clearly associated with the streaming of organelles, reticulation could disrupt the normal patterns of intracellular motility to an extent sufficient to cause aggregation. However, further clarification of the processes underlying reticulation and aggregation must await the identification of the nature of the cortical fibers in *Vaucheria*. Evidence that these fibers consist of large bundles of actin filaments is presented in Blatt and Briggs (1978) and the following paper (Blatt et al., 1980).

References

Åberg, H., Fries, L.: On cultivation of the alga *Vaucheria dichotoma* (Xanthophyceae) in axenic culture. Phycologia **15**, 133–141 (1976)

Blatt, M.R., Briggs, W.R.: A recording microphotometer for measurement of chloroplast orientation movements in single algal filaments. Carnegie Inst. Washington, Yearb. **76**, 278–281 (1977)

Blatt, M.R., Briggs, W.R.: Blue light-induced chloroplast aggregation and cortical fiber reticulation in the alga *Vaucheria*. Carnegie Inst. Washington, Yearb. **77**, 333–335 (1978)

Blatt, M.R., Wessells, N.K., Briggs, W.R.: Blue light induces cortical fiber reticulation concomitant with chloroplast aggregation in *Vaucheria sessilis*. Planta **147**, 363–375 (1980)

Fischer-Arnold, G.: Untersuchungen über die Chloroplastenbewegung bei *Vaucheria sessilis*. Protoplasma **56**, 495–520 (1963)

Goldman, R., Pollard, T., Rosenbaum, J. (eds.): Cell Motility, Cold Spring Harbor Conferences on Cell Proliferation, vol. 3. Cold Spring Harbor, N.Y., USA: CSH Lab. 1976

Haupt, W.: Localization of phytochrome in the cell. Physiol. Vég. **8**, 551–563 (1970)

Haupt, W., Schönbohm, E.: Light-oriented chloroplast movements. In: Photobiology of Microorganisms, pp. 283–307, P. Halldahl, ed. London: Wiley-Interscience 1970

Haupt, W., Schönfeld, I.: Über das Wirkungsspektrum der „negativen Phototaxis" der *Vaucheria*-Chloroplasten. Ber. Dtsch. Bot. Ges. **75**, 14–23 (1962)

Kamiya, N.: Protoplasmic streaming. In: Encyclopedia of Plant Physiology, Vol. XVII, pt. 2, pp. 979–1035, Ruhland, W., ed. Berlin, Heidelberg, New York: Springer 1962

Lechowski, Z.: Chloroplast arrangement as a factor of photosynthesis in multilayered leaves. Acta Soc. Bot. Pol. **43**, 531–540 (1974)

Mayer, F.: Lichtorientierte Chloroplasten-Verlagerung bei *Selaginella martensii*. Z. Bot. **52**, 346–381 (1964)

Ohiwa, T.: Response of *Spirogyra* chloroplast to local illumination. Planta **136**, 7–11 (1977)

Ott, D., Brown, M. Jr.: Developmental cytology of the genus *Vaucheria* I. Organization of the vegetative filament. Brit. Phycol. J. **9**, 111–126 (1974)

Schönbohm, E.: Untersuchungen über den Einfluß von Photosynthesehemmstoffen und Halogeniden auf die Starklicht- und Schwachlichtbewegung des Chloroplasten von *Mougeotia*. Z. Pflanzenphysiol. **60**, 255–269 (1969)

Schönbohm, E.: Kontraktile Fibrillen als aktive Elemente bei der Mechanik der Chloroplastenverlagerung. Ber. Dtsch. Bot. Ges. **86**, 407–422 (1973a)

Schönbohm, E.: Die lichtinduzierte Verankerung der Plastiden im cytoplasmatischen Wandbelag: eine phytochromgesteuerte Kurzzeitreaktion. Ber. Dtsch. Bot. Ges. **86**, 423–430 (1973b)

Schönbohm, E.: Der Einfluß von Colchicin sowie von Cytochalasin B auf fädige Plasmastrukturen, auf die Verankerung der Chloroplasten sowie auf die orientierte Chloroplastenbewegung. Ber. Dtsch. Bot. Ges. **88**, 211–224 (1975)

Seitz, K.: Zur Frage der Jodid-Wirkung auf die Starklichtbewegung der Chloroplasten von *Vallisneria spiralis* ssp. *torta*. Z. Pflanzenphysiol. **62**, 63–69 (1970)

Senn, G.: Die Gestalts- und Lageveränderung der Pflanzenchromatophoren. Leipzig: Engelmann 1908

Starr, R.: The culture collection of algae at Indiana University. Am. J. Bot. **51**, 1013–1044 (1964)

Virgin, H.: An action spectrum for the light induced changes in the viscosity of plant protoplasm. Physiol. Plant. **5**, 575–582 (1952)

Virgin, H.: Some effects of light on chloroplasts and plant protoplasm. In: Photophysiology, vol. I, pp. 273–303, Giese, A., ed. New York: Academic Press, 1964

Wagner, G., Haupt, W., Laux, A.: Reversible inhibition of chloroplast movement by cytochalasin B in the green alga *Mougeotia*. Science **176**, 808–809 (1972)

Wagner, G., Klein, K.: Differential effect of calcium on chloroplast movement in *Mougeotia*. Photochem. Photobiol. **27**, 137–140 (1978)

Waris, H.: The significance for algae of chelating substances in nutrient solutions. Physiol. Plant. **6**, 538–543 (1953)

Zurzycki, J.: Chloroplast arrangement as a factor in photosynthesis. Acta Soc. Bot. Pol. **24**, 27–63 (1955)

Zurzycki, J.: The mechanism of movement of plastids. In: Encyclopedia of Plant Physiology, vol. XVII, pt. 2, pp. 940–978, Ruhland, W., ed. Berlin, Heidelberg, New York: Springer 1962

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