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Photomovement in *Dunaliella salina*: Fluence rate-response curves and action spectra

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Abstract. We determined the action spectra of the photophobic responses as well as the phototactic response in *Dunaliella salina* (Volvocales) using both single cells and populations. The action spectra of the photophobic responses have maxima at 510 nm, the spectrum for phototaxis has a maximum at 450–460 nm. These action spectra are not compatible with the hypothesis that flavoproteins are the photoreceptor pigments, and we suggest that carotenoproteins or rhodopsins act as the photoreceptor pigments. We also conclude that the phototactic response in *Dunaliella* is an elementary response, quite independent of the step-up and step-down photophobic responses. We also determined the action spectra of the photoaccumulation response in populations of cells adapted to two different salt conditions. Both action spectra have a peak a 490 nm. The photoaccumulation response may be a complex response composed of the phototactic and photophobic responses. Blue or blue-green light does not elicit a photokinetic response in *Dunaliella*.

Key words: Action spectra (*Dunaliella*) – Blue and blue-green light responses – *Dunaliella* – Light-induced motile responses – Photophobic responses – Phototaxis

Introduction

Plants have a remarkable ability to sense the character of the external environment through the perception of light, thereby modifying their behavior, growth or development in order to respond to environmental conditions appropriately. At the cellular level, light affects various motile phenomena. These include chloroplast movement (for reviews see Haupt 1982; Zurzycki 1980) and cytoplasmic viscosity (Virgin 1951), responses that help ensure efficient levels of photosynthetic activity. Numerous and diverse motile responses to light are found in flagellated algal cells; they allow the organisms to find the optimal position for photosynthetic efficiency in their environment (for reviews see Nultsch 1980; Häder 1988).

The inventory of photomotile responses of flagellated cells includes 1) the phototactic response, in which the cells sense the direction of light and swim toward it; 2) the photophobic responses, in which the cells sense a change in the level of irradiance with respect to time and either make an apparent stop or a 90° turn, depending on whether there is an increase (step-up) or a decrease (step-down) in the light fluence, respectively; and 3) the photokinetic response, in which the swimming speed is influenced by light. The ultimate goal of our research is to elucidate the steps involved in the signal-transduction chains that couple the light stimuli with the various elementary responses in the wall-less, unicellular, motile, biflagellate Volvocalean alga, *Dunaliella*. However, there is some question as to what is an elementary response and what is a complex response composed of repetitive elementary responses (for a review of the arguments see Colombetti and Petracchi 1989). For example, Diehn (1980) considers that the positive phototactic response in *Euglena* is not an elementary response but is just a manifestation of a series of step-down photophobic responses, where the cell responds to a decrease in irradiance by undergoing a 90° turn. In this way the cell tracks the light direction by turning every time it begins to swim away from the light. Similarly, Schletz (1976) suggests that the phototactic response in *Volvox* may not be an elementary response, but can be explained as a result of a series of step-up photophobic responses, where the cilia on the lighted side respond to an increase in light and stop beating. Consequently, the colony turns toward the light.

As a first step in unraveling the elements of the signal-transduction chains that lead to the various light-activated motile responses in *Dunaliella*, we used action

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Note: Diagrams of the optical set-ups used for measuring the responses at the single-cell level and of the plans for building the phototaxometer described in this paper are available to the interested reader

spectroscopy to establish whether or not a response is an elementary response, and to identify the possible photoreceptor pigments that may activate the responses. In the present paper, we characterize the spectral requirements (380–600 nm) of the step-up photophobic, the step-down photophobic and the phototactic responses of *Dunaliella* using studies with single cells that are facilitated by computer-assisted video microscopy. We show that the phototactic response in *Dunaliella* is an elementary response and not based on photophobic responses as found in *Euglena* and *Volvox* and extrapolated to other organisms. We also characterize the spectral requirements of the photoaccumulation response of populations and suggest that the photoaccumulation response may be a complex response composed of two or more of the above elementary responses.

Material and methods

Culture of Dunaliella cells. Unless otherwise stated, *Dunaliella salina* (Dunal.) Teod. (strain 1644 from the University of Texas Culture Collection, Austin, USA) was grown to a density of about $5 \cdot 10^5$ cells $\cdot$ ml^{-1} (mid-logarithmic phase) in aerated modified AS100 medium (J. Brand, Botany Department, University of Texas, Austin, USA; personal communication). The modified medium included $MgSO_4$, 9.9 mM; KCl, 8.05 mM; $NaNO_3$, 11.77 mM; $CaCl_2$, 2.05 mM; 2-amino-2-(hydroxymethol)-1,3-propanediol (Tris base), 8.26 mM; KH_2PO_4, 367 µM; H_3BO_3, 554 µM; H_2SO_4, 180 µM; $ZnCl_2$, 2.31 µM; Na_2EDTA, 161.16 µM; $FeCl_3$, 17.98 µM; $(NH_4)_6Mo_7O_{24}$, 252 nM; $CoCl_2$, 5 nM; $MnCl_2$, 2 nM, and 10% NaCl. The medium was titrated with HCl to pH 8.08. The cells were grown in growth chambers (model MIR–150; Sanyo Electric Tokki, Tokyo, Japan) at 28° C under continuous irradiation from fluorescent lamps (model FL20SS $\cdot$ W/18; Toshiba Lighting and Technology, Tokyo) with an energy fluence rate of 8.6 W $\cdot$ m^{-2} as measured with a United Detector Technology (Santa Monica, Cal. USA) model 81; Optometer equipped with a radiometric sensor.

A subpopulation of the same strain of cells, which was conditioned over many years to grow on a low-salt medium, was obtained from Dr. K. Hara (Tsukuba University, Tsukuba, Japan). These cells were grown to a density of $5 \cdot 10^5$ cells $\cdot$ ml^{-1} (mid-logarithmic phase) in GPM medium (Loeblich 1975) at 25° C under the same continuous irradiation as the strain-1644 cells. The GPM medium includes 75% sea water (approx. 2.3% NaCl).

Measurement of photoaccumulation. In large-scale qualitative experiments, 200 ml of cells were placed in clear plastic rectangular chambers, and the monochromatic light from the Okazaki Large Spectrograph, National Institute for Basic Biology, Okazaki, Japan (Watanabe et al. 1982) was focused on various levels with two double convex lenses (see Fig. 1). The energy fluence rate was measured with an Epply (Newport, R.I., USA) temperature-compensated thermocouple connected to a nanovoltmeter (TR 8513B; Takeda Riken, Tokyo). Photoaccumulation, in this paper, is defined as the accumulation of a population of cells in the region of the chamber closest to the light source when the light beam is parallel to the long axis of the chamber.

For the small-scale, quantitative experiments, monochromatic light (spectral full width at half maximum = 6 nm) was obtained from the spectrograph using a quartz optical light guide. Photon fluence rates were varied by inserting custom-made neutral-density filters into the light path. Photon fluence rates were measured with a photon density meter (model HK–1; RIKEN, Saitama, Japan). In these experiments, 269 µl of cells ($5 \cdot 10^5$ cells ml^{-1}) were placed in a glass cuvette (38 mm long, 3 mm in diameter) that was immediately inserted in a phototaxometer, similar to that designed by Feinleib and Curry (1967). Briefly, the measuring beams were produced by red-light-emitting diodes (Radio Shack, Fort Worth, Tex., USA) placed perpendicular to, and at the front and back of the cuvette. The red beams were directed to the opposite side of the cuvette, where two cadmium sulfide (CdS) cells were placed. The changes in the resistances of the two CdS cells were amplified through two operational amplifiers. The outputs of these amplifiers were then compared and the voltage difference was recorded. Photoaccumulation, which causes an increase in optical density on the lighted side and a decrease in optical density on the shaded side of the cuvette, was recorded on a pen recorder (type 3066; Yokogawa Electric Works, Tokyo) and the rate of photoaccumulation was determined from the phototaxometer output in mV $\cdot$ min^{-1}. During an experiment the cells were shielded from all light except the actinic light. Experiments usually lasted between 1 and 2 min and were performed at 28° C under a dim red safelight. The photon fluence rates were corrected for the transmittance of the glass cuvette and the cell suspension by measuring the relative transmittance with a Hitachi (Tokyo) 557 spectrophotometer. The fluorescence of the cells and cuvettes as measured with a Hitachi MPF–4 fluorescence spectrophotometer was negligible (data not shown).

The tracker system. The tracker system is a computer-assisted video microscope that records, digitizes and displays the swimming tracks of approx. 50 cells per field (Kondo et al. 1988). The tracker system consists of a 2.54-cm silicon vidicon camera (C 1000–2; Hamamatsu Photonics, Hamamatsu, Shizuoka, Japan). The camera control unit includes a Hamamatsu M 1005 binarization board and a Hamamatsu M 998 I/O buffer. The digitized images (National Television Systems Committee fields) are fed into a BIWAC DMS–808 computer (Digi-Tek Laboratory, Ootsu, Shiga, Japan) every 1/60 s. Depending on the experiment, 60 or 120 fields are combined into 1 superfield and 8–16 superfields are collected. Each superfield represented 1 or 2 s. The superfields are printed consecutively with a color printer (Model G 500, Mitsubishi Electric Corp., Tokyo; or Model JP 80; Seiko Epson Corp., Shiojiri, Nagano, Japan). The video-processed image is also viewed on a graphics display terminal (Model JCC–M1401 II; Japan Computer Corp., Tokyo). Two hundred megabytes of superfield data were collected and analyzed for the experiments reported here. Only cells that remained in the field for the entire experiment were analyzed.

The video camera was attached to an inverted microscope (IMT–2; Olympus Corp., Tokyo) equipped with bright-field optics. The infrared observation beam originated from a 12 V/50 W tungsten-halogen lamp and was passed through a Hoya (Tokyo) IR 83 glass filter. The heat filter was removed from the collecting lens apparatus. The light was then passed through a phase-contrast condenser with an ultra-long working distance (numerical aperture (N.A) = 0.3). The cells were observed with a 4× SPLAN PL objective lens (N.A. = 0.13), a 1.5× optivar and a 5× projection lens. All the lenses are commercially available (Olympus, Tokyo). A custom-built electronic shutter, controlled by the computer, was mounted on the microscope in order to regulate the actinic monochromatic light. The line voltage was filtered with a stabilized D.C. power supply (Model PAB 32–2; Kikusui Electronics Corp., Kawasaki, Kanagawa, Japan) before it was connected to the microscope.

The monochromatic light (spectral full width at half-maximum approx. 1 nm) used for the single-cell experiments was obtained from the spectrograph. The photon fluence rate was measured with the HK–1 photon density meter (RIKEN). During the experiments, the cells were shielded so that they were only exposed to actinic light and the infrared observation beam.

For single-cell experiments the cells were placed in 15 mm wide, 10 mm long, 0.17 mm deep optically clear chambers. The chambers were covered with a cover glass to prevent the entry of oxygen and the occurrence of oxygen taxis. The imaging light was focused on cells in the center of the chamber.

Some of the experiments reported here were repeated on a Zeiss (Micro-Med Instruments, Walden, NY, USA) IM 35 inverted microscope connected to a commercially available Motion Analysis video processing and analyzing system (Motion Analysis Corp., Santa Rosa, Cal., USA). In this case the optical path included a

Plan 10× (N.A. = 0.22) objective, a long-working-distance condenser (N.A. = 0.63) and a 3.2× projection lens. The image was captured with a CCD camera (TI 23A; Nippon Electric Corp., Tokyo). The image was then digitized with a Motion Analysis VP 110 video processor (Motion Analysis Corp) and analyzed with an Amdek System 386 microcomputer (Amdek, San Jose, Cal., USA). In these experiments, video fields were collected every 1/30 s. The monochromatic light in these experiments was obtained from a DC-regulated 100-W quartz halogen source (Model 77503; Oriel Corp., Stratford, Conn., USA) combined with a continuously variable (400–700 nm) interference filter. The light was transmitted through a 9-mm glass optical light guide (Oriel) directly to the cell chamber through a custom-built stage.

Measurement of the step-up photophobic response. The step-up photophobic response was assayed by collecting seven superfields, each representing 1 s. Approximately 20000 single cells were analyzed.

Measurement of the step-down photophobic response. The step-down photophobic response was assayed by collecting 16 superfields. Each superfield represents 1 s. Approximately 30000 single cells were analyzed.

Measurement of the phototactic response. The phototactic orientation response was assayed by collecting six superfields, each representing 2 s. Ninety degrees represents movement toward the light source; zero degrees represents either a random orientation or movement perpendicular to the light source. Minus ninety degrees represents movement directly away from the light source. The cell paths were recorded with a digitizer (KD 4030; Graphtec Co., Tokyo). The angles were then determined with the aid of a microcomputer (PC980/VM2; Nippon Electric Corp., Tokyo). Approximately 10000 cells were analyzed.

Measurement of the photokinetic response. The effect of light on swimming speed or photokinesis was analyzed by determining the distance the cell traveled 0–2 s and 2–4 s after the onset of a directional light stimulus. The same digitized cell paths that were digitized by a naive person in order to determine the phototactic orientation response were used. The distance traveled was determined automatically by the NEC microcomputer. Approximately 10 000 cells were analyzed.

Determination of the absorption spectrum of living Dunaliella cells. The in-vivo absorption spectrum was determined by centrifuging cells to a concentration of approx. 10^7 cells · ml^{-1} and measuring their absorption with a custom-made spectrophotometer (Hitachi, Tokyo). Identical absorption spectra are obtained with a commercially available diode array spectrophotometer (model 8452A; Hewlett-Packard, Sunnyvale, Cal., USA).

A note on the analysis of the fluence rate-response curves. We do not present action spectra obtained from the "threshold" method of analyzing fluence rate-response curves proposed by Foster and Smyth (1980) because it was not possible to determine exactly where the linear portion of the fluence rate-response curves began and ended. A small error in determining the linear portion of the curve will result in a large error in extrapolating back to the threshold. The uncertainty in determining the beginning of the linear portion is a result, in part, of the large biological variation that occurs at low stimulus levels. For this reason, it is more reliable to use the traditional method where the photon fluence rates that give a 50% response are used to construct the action spectra (Galland 1987). While the action spectra obtained by the two methods are not substantially different, the "threshold" spectra are noisier (data not shown). The photon fluence rates needed to give the 50% points were determined from linear regressions using MINITAB (Minitab, State College, Penn., USA).

The half-maximal value used to construct each action spectrum is derived from the most effective wavelength since the photon fluence rate of the light was not high enough to obtain a maximal response for all wavelengths. In order to determine the relative quantum effectiveness of wavelengths whose photon fluence rate was not high enough to reach the half-maximum level of the most effective wavelength, we drew a line that originated from the highest obtainable fluence-rate point. The slope of this line was drawn the same as the slope of the fluence rate-response curve of the most effective wavelength for a given response. The point where this extrapolated line intersected the half-maximal value was used to construct the action spectrum. Thus, in the action spectra, the near-zero values given to these relatively ineffective wavelengths are maximal possible values.

Results

Photoaccumulation at the population level. The cells of *Dunaliella salina* (UTEX 1644) always swim toward the direction of the light source and not toward the direction of highest intensity. This is true whether monochromatic light (450–490 nm) is focused so that the intensity is greatest at the front of the chamber (Fig. 1b), the back of the chamber (Fig. 1d) or the middle of the chamber (Fig. 1e). The cells even swim through the region of highest light and subsequently accumulate against the wall of the chamber closest to the light source. Therefore the cells of *Dunaliella* are able to sense the direction and not the gradient of light – results that once more confirm the results obtained by Buder (1917). The cells of *Dunaliella* always accumulate against the wall closest to the light source and never accumulate against the wall fur-

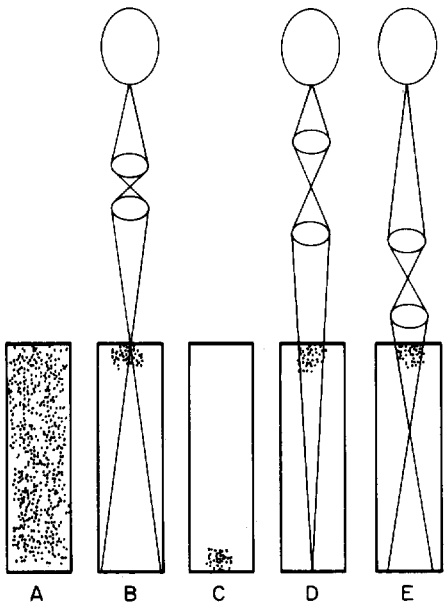

Fig. 1A–E. Qualitative demonstration of the behavior of a population of *Dunaliella* cells. **A** position of the cells in darkness; **B** final position of the cells after irradiation focused on the front wall of the chamber; **C** position of cells after they were irradiated as in B, but then were turned around before they were subjected to additional irradiation; **D** final position of cells that were originally in position **A** or **C**, and then subjected to irradiation focused on the back wall of the chamber; **E** final position of cells that were originally in position **A** or **C**, and then were subjected to irradiation focused in the middle of the chamber

thest away from the light source, even when we irradiate the cells with monochromatic light at energy fluence rates as high as $3.3 \cdot 10^{17}$ photons $\cdot$ cm$^{-2} \cdot$ s^{-1} (1350 W $\cdot$ m^{-2} at 490 nm). This contrasts with the results of Halldal (1958) whose strains of *Dunaliella* also exhibited a negative phototactic response.

Photomotile responses at the cell level. To confirm and to quantify these results and to separate clearly phototaxis from the other photoresponses (i.e., photokinesis, step-up and step-down photophobic responses), we investigated the effects of light at the single-cell level using computer-assisted video microscopy. Figure 2 shows a sample of cell tracks for the step-up photophobic response, the phototactic response and the step-down photophobic response, as well as a track of a cell swimming in the dark. The step-up photophobic response is defined as a decrease in the forward linear velocity of 50% or more within 1 s after the onset of irradiation. The step-down photophobic response is defined as a 90° or more change in swimming direction within 1 s following the termination of irradiation. The phototactic response is quantified as the angle subtended by the cell path and an imaginary line, perpendicular to the light source, at specified times after the onset of irradiation. Photokinesis is quantified by determining the distance traveled by a swimming cell 2–4 s after the onset of irradiation. Only the phototactic response is dependent on the direction of the light. The step-up photophobic, step-down photophobic and photokinetic responses are independent of light direction (Diehn et al. 1975).

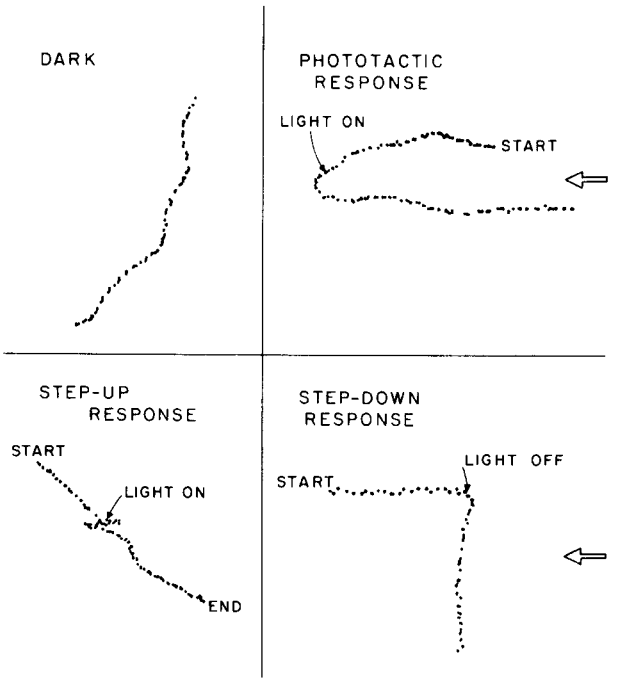

Fig. 2. Representative tracks of *Dunaliella* cells captured with the Motion Analysis System. Each dot represents the position of a single cell at intervals of 1/30 s. In the case of the phototactic response, the cell was irradiated with 450-nm light. In the cases of the step-up and step-down photophobic responses, the cells were irradiated with 510-nm light. The *large arrows* indicate the light direction and the *small arrows* indicate the time when the light conditions were changed

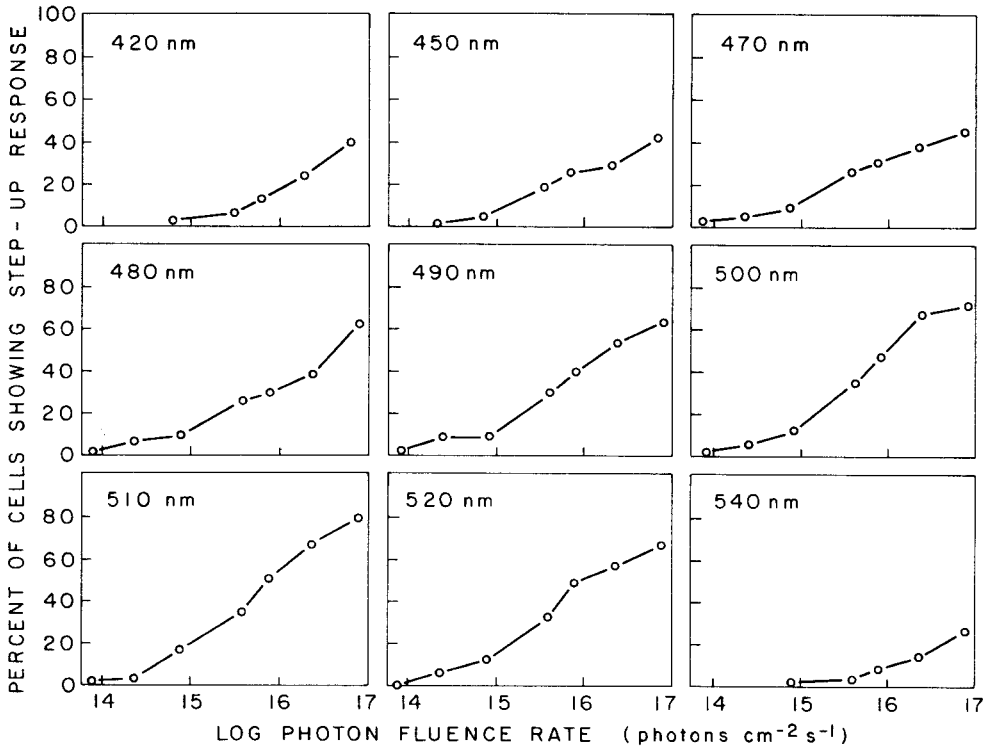

Fig. 3. Fluence rate-response curves for the step-up photophobic response in *Dunaliella*. The symbols represent the mean $\pm$ SE. The SEs are smaller than the symbols. Approximately 20 000 cells were analyzed

Step-up photophobic response. When the cells swimming in the dark are subjected to a nearly instantaneous increase in the photon fluence rate, the cells exhibit a step-up photophobic response. The decrease in the forward velocity results from the cells momentarily changing from forward to backward swimming behavior. This is a consequence of a transient (several 100 ms) change from the ciliary waveform to the flagellar waveform (data not shown). The step-up photophobic response requires rather high photon fluence rates. The percentage of cells showing a step-up photophobic response is proportional to the logarithm of the photon fluence rate from 10^{15} to 10^{17} photons $\cdot$ cm$^{-2} \cdot$ s^{-1} (Fig. 3). The value of the photon fluence rate that is required to obtain a half-maximal response (40% of the cells showing a step-up photophobic response) was used to generate an action spectrum, which has a peak at 510 nm and a spectral full width at half maximum of 34 nm (Fig. 4).

Step-down photophobic response. Following the termination of irradiation, cells immediately turn at least 90°. The percentage of cells that exhibit the step-down response is approximately proportional to the log of the photon fluence rate from 10^{14} to 10^{16} pho-

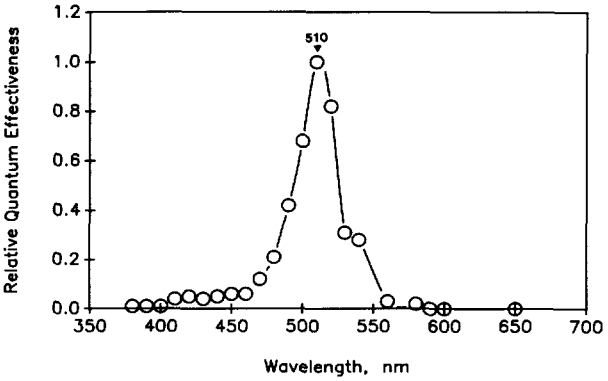

Fig. 4. Action spectrum for the step-up photophobic response in *Dunaliella*. The action spectrum is derived from the photon fluence rates required for a half-maximal response (40% of the cells showing a step-up photophobic response). Their reciprocals are plotted against wavelengths. The curve is normalized to 510 nm

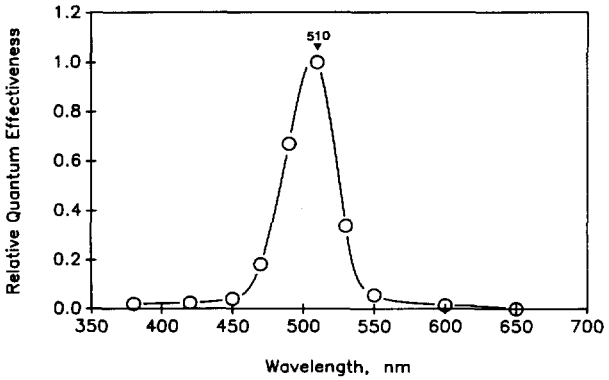

Fig. 6. Action spectrum for the step-down photophobic response in *Dunaliella*. The action spectrum is derived from the photon fluence rates required for a half-maximal response (30% of the cells showing a step-down photophobic response). Their reciprocals are plotted against wavelengths. The curve is normalized to 510 nm

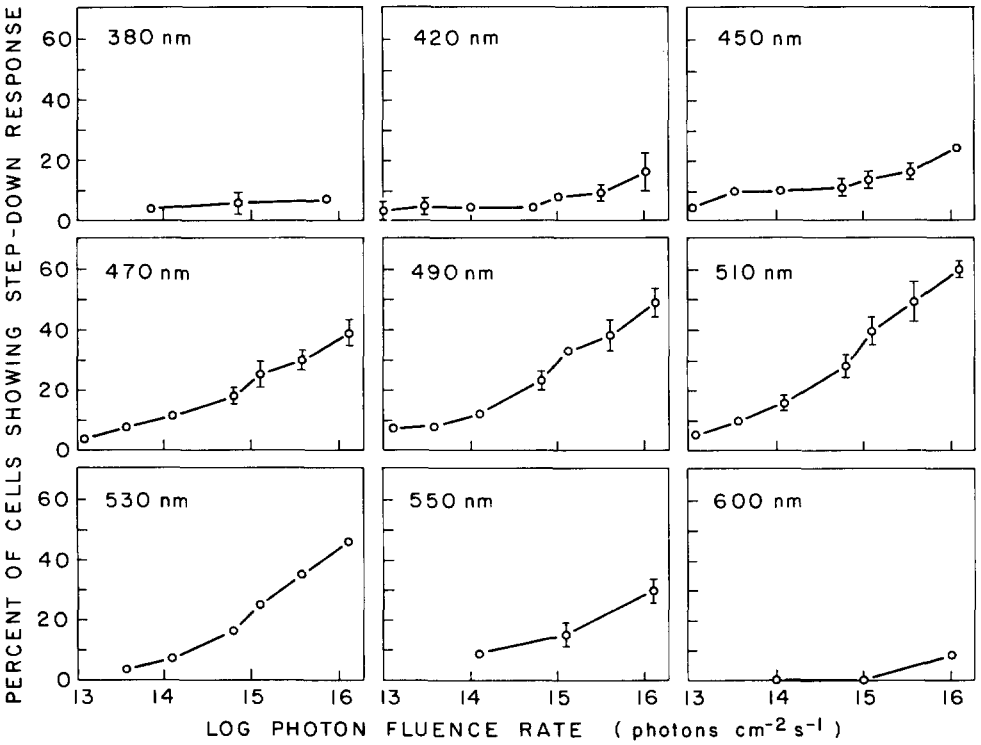

Fig. 5. Fluence rate-response curves for the step-down photophobic response in *Dunaliella*. The symbols represent the mean $\pm$ SE. Approximately 30 000 cells were analyzed

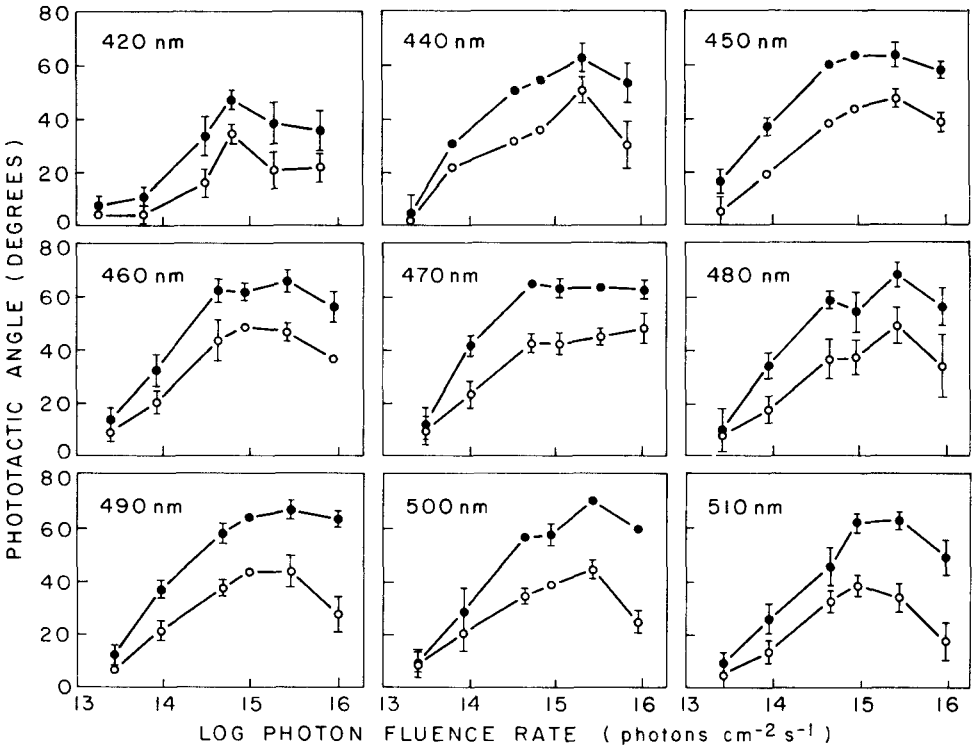

Fig. 7. Fluence rate-response curves for the phototactic response in *Dunaliella* measured over the intervals between 0–2 s (○) and 2–4 s (●) after the onset of irradiation. Ninety degrees represents movement toward the light source; zero degrees represents either a random orientation or movement perpendicular to the light source. Minus ninety degrees represents movement directly away from the light source. The symbols represent the mean ± SE

tons · cm^{-2} · s^{-1} (Fig. 5). The value of the photon fluence rate required to obtain a half-maximal response (30% of the cells showing a step-down photophobic response) was used to generate an action spectrum (Fig. 6), which has a peak at 510 nm and a spectral full width at half-maximum of 40 nm.

Phototactic response. When the cells swimming in the dark are subjected to a directional light stimulus, the cells exhibit a phototactic response. The cells immediately and smoothly turn and swim toward the light source. The cells only exhibit a positive phototactic response. Saturation of the phototactic response requires lower photon fluence rates than the saturation of the step-up photophobic response. The mean angle of the cell paths increases with the log of the photon fluence rate from 10^{13} to 10^{15} photons · cm^{-2} · s^{-1} (Fig. 7) in a manner consistent with an assumed linearity. Above 10^{15} photons cm^{-2} · s^{-1} the phototactic response decreases as the photon fluence rate increases. This is especially true for the time interval between 0–2 s after the onset of irradiation (see below). The values of the photon fluence rate that is required to obtain a half-maximal response for the first (0–2 s) and second (2–4 s) time period was used to generate action spectra, which have peaks at 450 and 460 nm, respectively, and spectral full widths at half-maximum of 80 nm (Fig. 8).

In order to characterize the phototactic response further, we determined the distance traveled by the cells for the first 2 s and the second 2 s after the onset of the directional irradiation. Figure 9 shows that during the first 2 s light causes a decrease in the distance traveled and the distance decreases approximately linearly with

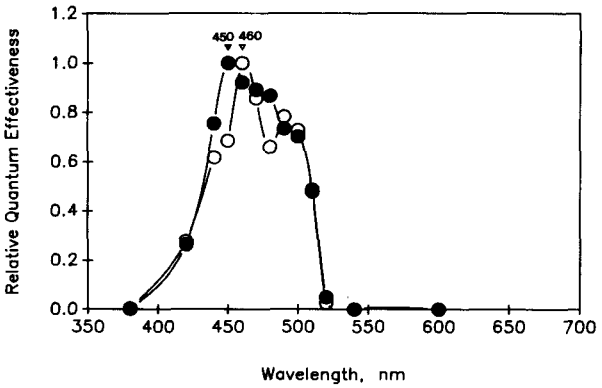

Fig. 8. Action spectrum for the phototactic response in *Dunaliella*. The action spectrum is derived from the photon fluence rates required for a half-maximal response (25° for 0–2 s after the onset of irradiation (○) and 30° for 2–4 s after the onset of irradiation (●). Their reciprocals are plotted against wavelengths. The curves are normalized to 460 and 450 nm, for the period 0–2 s and 2–4 s after the onset of irradiation, respectively

the logarithm of the photon fluence rate from about 10^{14} to 10^{16} photons · cm^{-2} · s^{-1}. Notice that the slope of the curves depends on wavelength. We constructed an action spectrum from these fluence rate-response curves. We linearly extrapolated each fluence rate-response curve in order to determine the photon fluence rate at each wavelength needed to decrease the distance traveled to 50 μm. The action spectrum has a major peak at 520 nm and a minor peak at 460 nm (Fig. 10). The spectral full width at half-maximum of the major peak is 40 nm.

In contrast to the inhibitory effect of light on the distance traveled over 0–2 s after the onset of directional

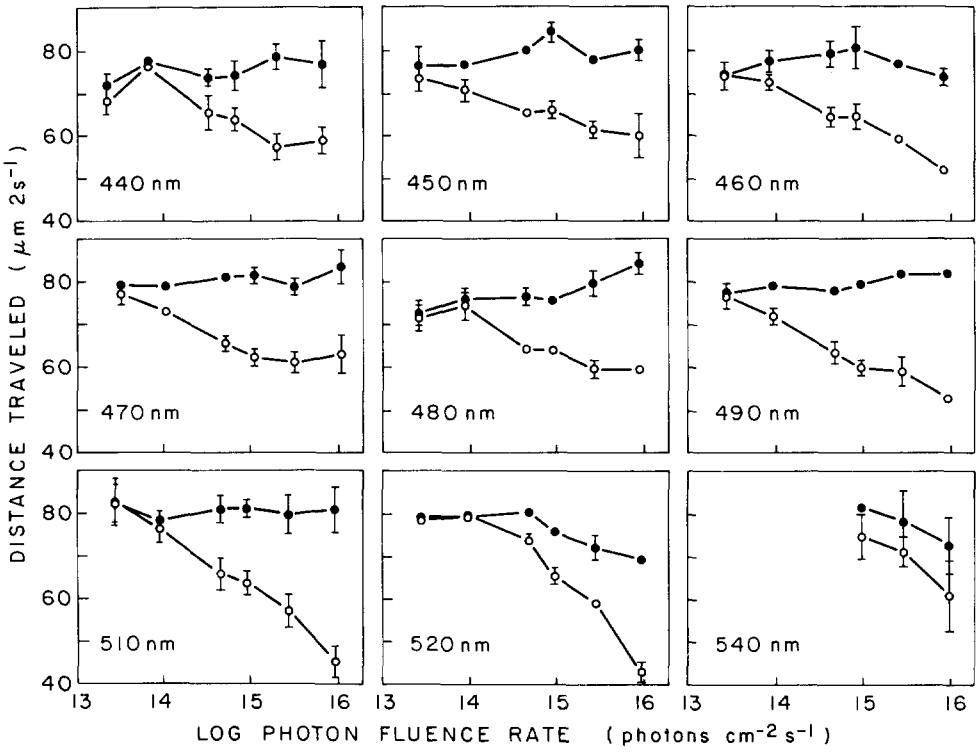

Fig. 9. Fluence rate-response curves for the distance traveled over 2 s in *Dunaliella* measured over the intervals between 0–2 s (○) and 2–4 s (●) after the onset of irradiation. The symbols represent the mean ± SE

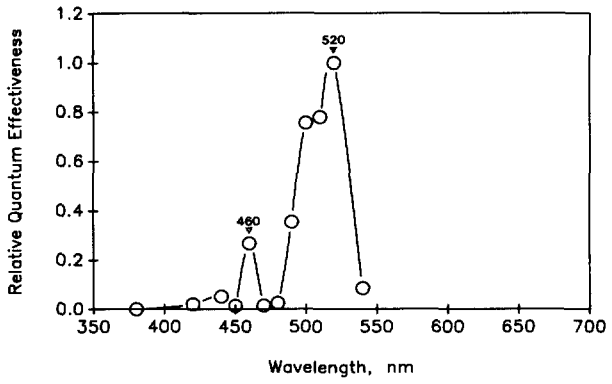

Fig. 10. Action spectrum for the inhibitory effect of light on the distance traveled over the period 0–2 s after the onset of irradiation in *Dunaliella*. The action spectrum is derived from linearly extrapolating the fluence-response curves to 50 μm · s^{-1} and plotting the reciprocal of the photon fluence rates required to reduce the distance traveled to 50 μm · $(2s)^{-1}$ against wavelengths. The curve is normalized to 520 nm

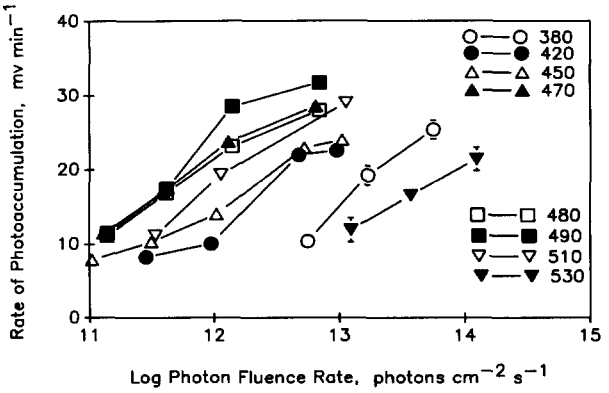

Fig. 11. Fluence rate-response curves for the photoaccumulation response in *Dunaliella* grown in 10% NaCl. The symbols represent the mean ± SE

irradiation, blue-green light has no effect on the distance traveled 2–4 s after the onset of irradiation (Fig. 9). In fact, the distance traveled is the same as that traveled in darkness (data not shown). These data indicate that photokinesis in *Dunaliella* does not exist with photon fluence rates of up to 10^{16} photons · cm^{-2} · s^{-1} over the spectral range of 380–540 nm.

Photoaccumulation. Once we determined the action spectra of the individual photomotile responses of *Dunaliella*, we decided to see if only the phototactic response is responsible for the photoaccumulation of a population of cells near a light source or if the photoaccumulation response is a complex response composed of more than one photoresponse. The accumulation response of a population of cells can be quantified with the aid of a phototaxometer. Using the phototaxometer, the photoaccumulation response shows no perceptable (< 1 s) lag time (data not shown). The rate of photoaccumulation is approximately proportional to the logarithm of the photon fluence rate (Fig. 11). *Dunaliella* cells are highly sensitive to blue-green light and very low photon fluence rates; 10^{13} photons · cm^{-2} · s^{-1} are required to saturate the photoaccumulation response. The value of the photon fluence rate required to obtain a half-maximal response (16.5 mV · min^{-1}) was used to generate an action spectrum (Fig. 12), which has a broad peak at

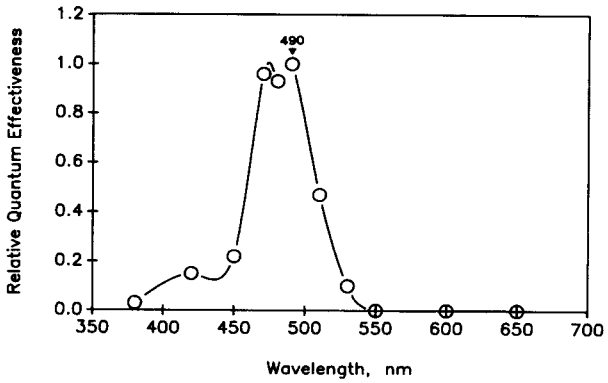

Fig. 12. Action spectrum for the photoaccumulation response in *Dunaliella* grown in 10% NaCl. The action spectrum is derived from the photon fluence rates required for a half-maximal response (16.5 mV · min^{-1}). Their reciprocals are plotted against wavelengths. The curve is normalized to 490 nm

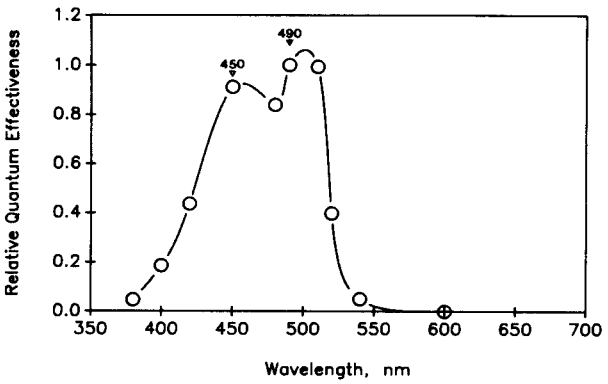

Fig. 13. Action spectrum for the photoaccumulation response in *Dunaliella* grown in 2.3% NaCl. The action spectrum is derived from the photon fluence rates required for a half-maximal response (16.5 mV · min^{-1}). Their reciprocals are plotted against wavelengths. The curve is normalized to 490 nm

490 nm and a spectral full width at half-maximum of 50 nm.

Since our strain of *Dunaliella salina* typically grows in a 10% NaCl medium, we repeated our determination of the fluence rate-response curves and action spectrum for the photoaccumulation response using a subpopulation of our strain that had been conditioned to grow on a low-salt medium (approx. 2.3% NaCl) over a number of years. When the same type of experiment as in Fig. 11 is done with low-NaCl cells, it is found that the rate of photoaccumulation in these cells is also approximately proportional to the logarithm of the photon fluence rate (data not shown). The response is saturated at about $3 \cdot 10^{13}$ photons · cm^{-2} · s^{-1}. From these data the action spectrum shown in Fig. 13 was generated. The action spectrum has a broad peak at 490 nm, a shoulder at 450 nm and a spectral full width at half-maximum of 105 nm. We do not know the mechanisms responsible for the broadening of the action spectrum for photoaccumulation in the low-salt-conditioned cells.

Absorption spectrum of cells. In order to characterize the possible screening pigments, we obtained an absorption spectrum of a living population of *Dunaliella* cells. Figure 14 shows that the absorption spectrum results primarily from absorption by the photosynthetic pigments and there is no fine structure between 400 and 510 nm. Since our strain of *Dunaliella* has no stigma, no absorption spectrum of the stigma can be presented.

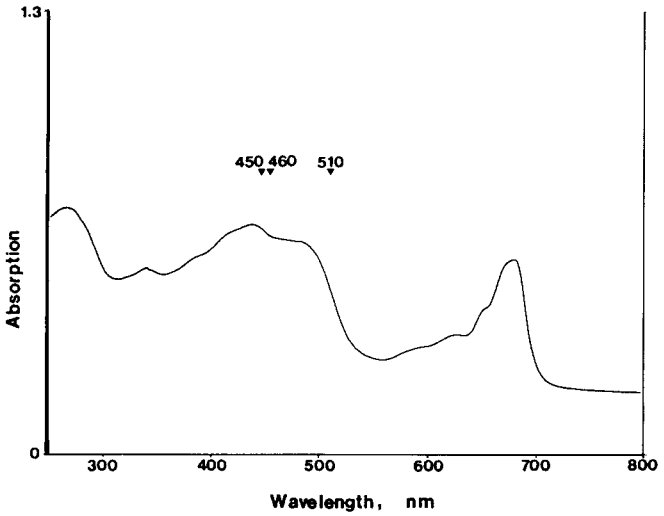

Fig. 14. Absorption spectrum of a dense population of *Dunaliella* cells. *Arrows* indicate 450 nm, 460 nm, and 510 nm, the peaks of the action spectra for the phototactic and photophobic responses

Discussion

Dunaliella exhibits a variety of blue- and blue-green-light-activated motile responses. The step-up photophobic and step-down photophobic responses of *Dunaliella salina* have action spectra which appear identical within the error range and which therefore indicate a common photoreceptor pigment. This photoreceptor pigment has a single peak around 510 nm. The identity of the photoreceptor pigment remains unknown; however, the action spectrum is inconsistent with a flavoprotein being the photoreceptor pigment in *Dunaliella*. By contrast, a flavoprotein is the photoreceptor pigment of the step-down photophobic response in *Euglena* (Barghigiani et al. 1979). Moreover, potassium iodide, a quencher of the triplet state of flavins, at concentrations as high as 200 mM (1–120 min) has no effect on the step-up photophobic and step-down photophobic responses in *Dunaliella* (data not shown). Again this is different from the effect of KI on the step-down photophobic response of *Euglena* (Diehn and Kint 1970; Mikolajczyk and Diehn 1975). Wavelengths between 500 and 600 nm were shown to be the most effective in some light responses in algal cells, including germination in *Scrippsiella* (Binder and Anderson 1986), translational regulation of protein synthesis in *Volvox* (Kirk and Kirk 1985), rhodopsin synthesis in *Chlamydomonas* (Foster et al. 1988) and the rapid photoelectric effect in *Acetabularia* (Schilde 1968). The photoreceptor pigment in these responses may be a carotenoprotein, or even a rhodopsin-like molecule (Foster et al. 1984).

The phototactic response of *Dunaliella* has a very different action spectrum. It has a peak around 450–460 nm and a shoulder around 470–490 nm. The main peak is similar to the peaks of action spectra of blue-light responses in which flavoproteins are favored for the photoreceptor. However, there are some distinct differences. Firstly, wavelengths in the green region of the spectrum, including 500 and 510 nm, are substantially more effective in the phototactic response than in blue-light responses and secondly, there is little activity at 380 nm and 420 nm, two wavelengths that are effective in initiating the typical blue-light responses (Senger and Lipson 1987). In addition, using a custom-made Hitachi Fourier transform microspectrofluorimeter (Matsui et al. 1989), we were unable to see any flavin fluorescence localized in or near the cilia (data not shown) as seen by Benedetti and Lenci (1977) and Kawai (1988) in other organisms. Furthermore, KI, at concentrations as high as 200 mM (1–120 min) has no effect on the phototactic response (data not shown). For these reasons, we believe that the photoreceptor pigment is not a flavoprotein. It is possible that the photoreceptor pigment responsible for the phototactic response is also a rhodopsin-like molecule or a carotenoprotein, although one different from what (those) responsible for initiating the photophobic responses. The action spectra of the phototactic response in *Dunaliella* are similar to the action spectra of the phototactic responses of *Gymnodinium* (Forward 1974) and *Porphyridium* (Nultsch 1980) as measured with single cells, indicating that the phototactic responses of these cells may share a common photoreceptor pigment.

The step-up photophobic response is the basis for the phototactic response in *Volvox* (Schletz 1976). The peaks of the action spectra for the phototactic and the step-up photophobic response are 491 and 480–490 nm, respectively. By adding the action spectrum of the step-up photophobic response to that of the absorption spectra of the screening pigment, Schletz was able to derive a combined spectrum that matches the action spectrum of the phototactic response. It can be seen by visually combining the action spectrum of the step-up photophobic response in *Dunaliella* (Fig. 4) with that of the absorption spectrum (Fig. 14), it is not possible to derive the action spectrum for the phototactic response unless we assume the absorption spectra of the shading pigments contributes more to the phototactic action spectrum than does the absorption spectrum of the photoreceptor pigment. We do not think that this is a reasonable assumption and conclude that the step-up photophobic response is not the basis of the phototactic response in *Dunaliella*. This interpretation is further supported by the differing ionic requirements of the phototactic and the photophobic responses (Noe and Wayne 1990).

While it has been proposed that the step-down photophobic response is the actual basis of the positive phototactic response in *Euglena* (Diehn 1980), this is unlikely to be the case in *Dunaliella* since the action spectra, the kinetics, and the ionic requirements of the phototactic response and the step-down photophobic responses are clearly distinct. It is clear from these considerations that single cells of *Dunaliella* are able to sense the light direction, and the phototactic response is an elementary response, and not the result of step-up or step-down photophobic responses. This is at variance with the opinion of many who extrapolate the well-established hypothesis for *Euglena* to other flagellates (see Colombetti and Petracchi 1989 for a comprehensive review of the arguments).

The ability of a single cell to undergo three specific elementary photoresponses activated by blue-green light and resulting from a change in the motility of the same organelles, the cilia, is quite remarkable. How can the cell undergo three different responses to a light stimulus? There must be differences in the receptors and-or the components, kinetics and-or spatial localization of the elements of the signal-transduction chains that activate these responses. The specificity of the photoresponses is derived, in part, from the heterogeneity at the level of the photoreceptor pigment. We are currently using the data derived from action spectroscopy, to activate each photoresponse separately in order to identify the components involved in the signal-transduction chains that lead to the elementary responses, and more importantly, understand how a single cell can execute three different movements in response to light using three different signal-transduction chains that have common components. The fact that *Dunaliella salina* is a wall-less, unicellular organism greatly facilitates electrophysiological, biochemical and physiological studies of the signal-transduction chains associated with light-stimulated responses.

Populations of *Dunaliella* accumulate in the light, presumably to optimize photosynthesis. We used a population method to characterize the spectral characteristics of the photoaccumulation response. The resulting action spectra are not similar to the action spectra of the phototactic or photophobic responses in *Dunaliella* measured with single-cell methods. It is possible that the action spectrum of photoaccumulation is a complex action spectrum that is the resultant of two or more action spectra of simpler responses, including the phototactic and photophobic responses. The phototactic response can contribute to photoaccumulation by directing distant cells toward the light. The step-up photophobic response may also participate in photoaccumulation by "holding" the cell once it arrives in the light and finally, the step-down photophobic response may also facilitate photoaccumulation by changing the swimming direction if the cell swims away from the light.

The action spectra of the photoaccumulation response presented here share similar characteristics with the action spectrum of "phototaxis" presented by Halldal (1958) for *Dunaliella* and other Volvocalean algae. All the action spectra obtained by the pioneering work of Halldal using the population method have peaks at 493 nm. The differences in the shapes of his action spectra and the action spectra presented here may result from the differences in the optical conditions employed in the different experiments. Halldal (1958) used an actinic light beam that was oriented perpendicular to the long axis of the cell chamber while we used an actinic light beam that was parallel to the long axis of the cell chamber. If, in

fact, both population methods measure the photoaccumulation response, which may be a complex behavioral response composed of simpler light-activated behavioral responses, then each experimental setup may activate each simple photoresponse to various degrees.

In conclusion, the phototactic response in *Dunaliella* is an elementary behavioral response, quite independent of the step-up and step-down photophobic responses, while photoaccumulation may be a complex response composed of the phototactic and photophobic responses. The action spectra indicate that the same photoreceptor pigment may be responsible for both photophobic responses whereas a second photoreceptor pigment is responsible for the phototactic response. It seems that neither of these pigments are flavoproteins. We suggest that they may be carotenoproteins or rhodopsins.

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