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Communication

Phytochrome Activation of K^+ Channels and Chloroplast Rotation in *Mougeotia*[1]

Action Spectra

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ABSTRACT

The action spectra for K^+ channel activation and chloroplast rotation are shown to be similar. Both phenomena exhibit activation at 660 nanometers, inhibition at 740 nanometers, and partial activation at 460 to 500 nanometers. This confirms that K^+ channels in *Mougeotia* are regulated by phytochrome, and indicates that both phenomena share at least part of the same transduction pathway.

Mougeotia is a model system for studies of phytochrome-mediated physiological processes. Research has concentrated on the effects of light upon the positioning of the single large chloroplast (5). The discovery that red light activates K^+ channels in the plasma membrane, activation that is reversed by far-red light (8), and that both chloroplast positioning (12) and K^+ channel activity (9) are also activated by Ca^{2+}, suggests that both may share part or all of the same transduction pathway. Phytochrome activation could lead to increases in cytosolic Ca^{2+} that in turn could directly activate the K^+ channel as well as induce chloroplast rotation.

Besides phytochrome, K^+ channel activity could also be regulated by photosynthesis, as indicated in *Elodea*, where K^+ uptake is activated by light and inhibited by DCMU (10), in *Nitella*, where there is a temporal correlation between Chl fluorescence and K^+ conductance (17), in *Riccia*, where light-induced DCMU-inhibited acidification of the cytoplasm is followed by an increase in cytoplasmic K^+ (for which the effect of DCMU was not determined) (1), and in *Eremosphaera*, where either a transition to dark or DCMU stimulates K^+ conductance (7). Therefore, photosynthesis could also regulate K^+ channel activity in *Mougeotia*.

In this paper, we present the action spectra for K^+ channel activation and chloroplast rotation. We show that K^+ channel activation matches the action spectra for phytochrome-mediated processes with no indication of regulation by photosynthesis. The similar action spectra for chloroplast rotation and K^+ channel in *Mougeotia* suggests that these two processes share at least in part the same transduction pathway.

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MATERIALS AND METHODS

Culturing and Protoplast Preparation

Mougeotia (UTEX LB 758 *Mougeotia* sp. [14]) was cultured according to Serlin and Ferrell (13), and protoplasts prepared as previously described (8) using an osmoticum/buffer solution containing 0.25 M mannitol, 0.25 M sorbitol, 2 mM $CaCl_2$, and 16 mM Mes (pH adjusted to 5.4 with KOH).

Ion Channel Measurements

Ion channel activity (3) of the *Mougeotia* plasma membrane was measured as previously described (8) with a patch clamp amplifier (model 8900, Dagan Corporation, Minneapolis, MN) and recorded on a pulse code modulated data recording system (model DAS-900, Dagan Corporation). Even with low resistance seals (less than 500 MΩ), there was little short-term noise, so channels with amplitudes greater than 0.25 pA were readily identified. In the experiments shown here, the channels open in the negative direction, whereas in previous work (8, 9) they are shown opening in the positive direction. This is due to different instrumentation. In either case, K^+ ions are moving into the cell.

When a stable seal was obtained, the microscope lamp and room lights were turned off. After 4 to 5 min of recording in the dark, the light source was turned on for a period of 30 s, then recording continued for 10 min.

For analysis, ion channel data were played back and digitized at 200 Hz (Labmaster DMA, Scientific Solutions, Cleveland, OH) after filtering at 40 Hz (−3 decibel cutoff) with an eight-pole Bessel filter (Frequency Devices, Haverhill, MA). The digitized data were analyzed using pClamp software (Axon Instruments, Burlingame, CA); the channel characteristics were analyzed from the pClamp compiled data files

using a statistical software package (SYSTAT Inc., Evanston, IL). The ratio of total channel open time before and after light irradiation was used to determine the extent of activation (9). The time period of measurement before and after irradiation was usually 5 min, but on occasion, shorter periods of time had to be used if the seal was lost. At least five experiments were performed for any particular wavelength. The data are shown either as extent of activation, or as the ratio of experiments showing activation to total experiments (this allowed direct comparison with chloroplast rotation data [see below]). Extent of activation was defined as the ratio of total channel open time after irradiation to total channel open time before irradiation. Channel activation was assumed to have occurred if the ratio was greater than 1.

Chloroplast Rotation

Chloroplast rotation was examined as described by Serlin and Ferrell (13) with the following modifications. After filaments were positioned in the plexiglass wells containing 10% osmoticum/buffer solution, they were kept in the dark for 40 min at 20 to 23°C. Filaments were then given a 30 s light irradiation and left in the dark for 35 min. Then chloroplast rotation was scored under green safe light; this was followed by a red light irradiation. The final red light irradiation was given to ensure that chloroplasts that had not rotated were indeed competent to rotate. Chloroplasts were scored as having rotated if they moved from edge view to face view. Data are shown as the ratio of the number of chloroplasts that rotated to total number of chloroplasts competent to rotate.

Light Irradiations

A monochromator (Mini-Chrom model 03, 4.4 nm bandwidth, Optometrics Corp., Ayer, MA) was inserted in the light path of a Nikon Optiphot microscope. The lamp (12 V, 100 W halogen bulb) was powered by a separate power supply outside of the Faraday cage to avoid contamination of records with 60 Hz noise. The light output through the monochromator was adjusted as follows. The monochromator exit slit was focused as closely as possible using a 10× objective by adjusting the Abbe condensor. This resulted in an irradiation area of 0.015 cm^2. A radiometer probe (detector model 268R, instrument model 350, United Detector Technology, Hawthorne, CA) was placed on the microscope sample stage and irradiance adjusted to 0.015 μW at the power supply to yield 1 $\mu W\ cm^{-2}$ irradiance. Radiant flux was maintained constant, rather than quantum flux, for the various wavelengths. Quantum flux varies less than twofold over this range.

RESULTS

K^+ Channel Activation

Time-compressed recordings of the channel data are shown in Figure 1 for irradiations at 500, 660, and 740 nm. Channel openings are seen as step-like transient downward shifts in current. About 2 min after irradiation with 660 nm light the amount of channel activity increased, with 500 nm light it remained fairly constant; whereas with 740 nm light channel activity clearly decreased. For all experiments channel ampli-

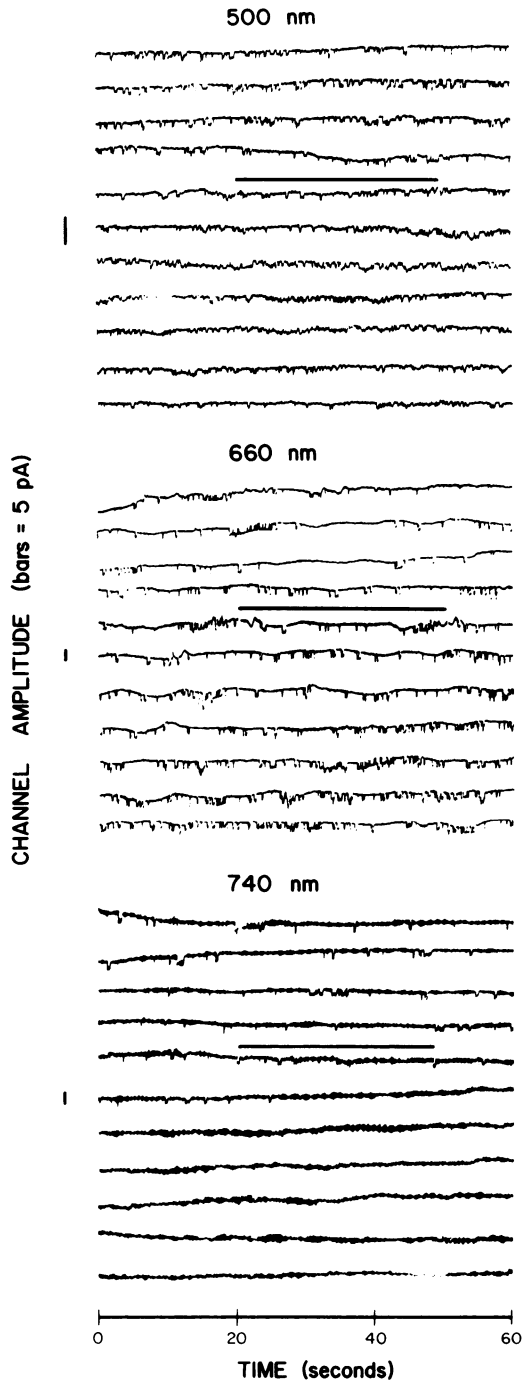

Figure 1. Channel activity in the plasma membrane of *Mougeotia*. Effect of 500, 660, and 740 nm light. Examples of channel activity are shown for light irradiations (500 nm, upper panel; 660 nm, middle panel; 740 nm, bottom panel, all at 1 $\mu W\ cm^{-2}$) given about 4.5 min after the beginning of the experiment (shown as a bar in the traces). A detailed description of channel behavior can be found in ref. 8. Channel activity, seen as step-like downwards shifts in the current, increases about 2 min after irradiation with 660 nm, remains fairly constant with 500 nm irradiation, and virtually disappears with 740 nm irradiation.

tudes ranged from about 0.3 to about 3.9 pA (mean, 1.42 [n = 60]) and median open times ranged from 23 to 320 ms (mean, 74 [n = 60]). Two channels, separable by their amplitudes, were occasionally seen as reported previously (8) (see refs. 8 and 9 for more detailed descriptions of the two channels). The direction of K^+ ion movement is into the cell from the pipette.

The action spectrum showing the results of each experiment at the various wavelengths is shown in the upper panel of Figure 2. Maximal activation is seen at 660 nm, strong inhibition at 740 nm, and some activation is seen at shorter wavelengths, especially around 460 to 540 nm. A significant number of outliers (16) are seen at most wavelengths except 660 and 740 nm. But the data clearly cluster, as shown by the medians and hinges (16).

A higher irradiation of 10 μW cm^{-2} was used at 500 nm to confirm the presence of blue light activation of K^+ channels. Indeed, a modest increase in activation was observed: in separate experiments, 0.17, 0.23, 0.49, 1.30, 1.36, and 1.56-fold increases in activity were seen (50% activation compared with 40% at 1 μW cm^{-2}). The median level of activation (0.894) was twofold greater than that at 1 μW cm^{-2} irradiance.

Chloroplast Rotation

The action spectra for chloroplast rotation and K^+ channel activation, both as the ratio of chloroplasts (or experiments) that rotated (or showed activation) to total number of chloroplasts competent to rotate (or total number of experiments) are shown in the bottom panel of Figure 2. Clear activation is seen at 660 nm; the extent of activation declines at shorter at shorter and longer wavelengths, although less steeply for chloroplast rotation compared with K^+ channel activation. For chloroplast rotation there was a smaller peak of rotation at 460 nm.

DISCUSSION

The measurements of K^+ channel activation by light are characterized by variability, apparent as the presence of outlier data points in the action spectrum (Fig. 2, upper panel). Even in the dark control, one experiment showed activation. Some possible explanations for the outliers include: (a) measurements are being performed on a single protoplast whose physiological state may differ from those of other protoplasts in the population; and (b) in the protoplast, we are measuring the activity of a single ion channel protein. In general, however, the data values clustered, and the activation by 660 nm light is quite clear.

To compare as directly as possible chloroplast rotation and K^+ channel activation, rotation was assayed using the same irradiation set-up. One significant difference that could not be resolved is the extent of dark adaptation prior to monochromatic light irradiation. For patch clamping, the seal must be formed in the light, and it would appear that the K^+ channels are activated to some extent while the seal is being formed because 740 nm light consistently caused inactivation. The two action spectra are similar to each other and to a previously reported action spectrum for chloroplast rotation (4), given the limitations of noise noted above. Both phenom-

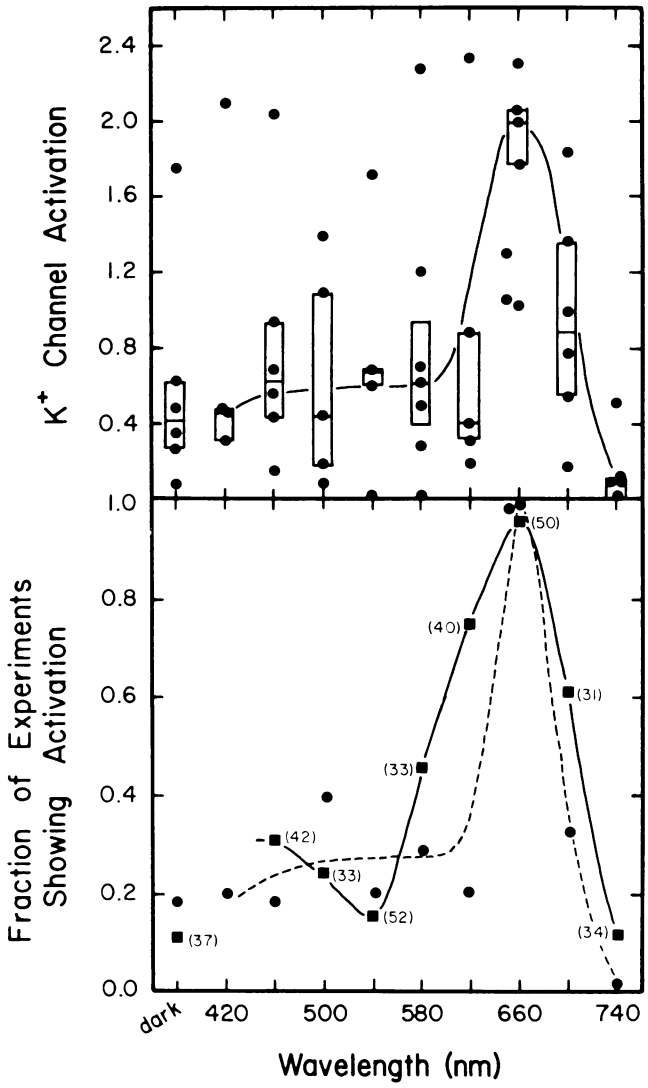

Figure 2. Action spectra for K^+ channel activation and chloroplast rotation in *Mougeotia*. Upper panel, The data for all K^+ channel experiments are shown to indicate the presence of outliers. Medians and hinges are shown to emphasize the clustering of the results. Activation was calculated as the ratio of total channel open time after to that before light irradiation. A ratio less than 1 indicates a decrease in channel activity, and a ratio greater than 1 indicates activation. For all wavelengths, radiant flux was constant at 1 μW cm^{-2}. Dark controls are also shown. Lower panel, The ratio of chloroplasts that rotated compared with total chloroplasts competent to rotate (solid squares, solid line; sample size is given next to each data point) is shown for the various wavelengths, as are the ratio of experiments that showed K^+ channel activation compared with total experiments for the various wavelengths (solid circles, dotted line). Dark controls are also shown.

ena are activated strongly by 660 nm light and show some activation at shorter wavelengths. The spectral peak of activation around 660 nm is narrower for channel activation compared with the peak for chloroplast rotation. In the case of chloroplast rotation, partial activation by blue light (4) is apparently caused by a second photoreceptor (2). Although we have not examined reversibility of blue light activation by far-red light, partial channel activation between 460 and 580 nm is presumably caused by the presence of the second photoreceptor.

Recent work examining light regulation of K^+ transport has implicated regulation by photosynthesis (1, 7, 10, 17). The action spectrum for K^+ channel activation in *Mougeotia* is inconsistent with regulation by photosynthetically active light at 1 μW cm^{-2} for 30 s. Given that photosynthesis is reported to deplete cytosolic Ca^{2+} (11), activation by photosynthesis is not expected because the K^+ channel is activated by Ca^{2+} (9). Furthermore, reversibility of red light-induced activation by far-red light (8) is inconsistent with activation by photosynthesis.

As is true in *Mougeotia,* K^+ channel activity and chloroplast movements are induced by light and Ca^{2+} in *Eremosphaera viridis,* although phytochrome is not implicated. Chloroplast translocation (systrophe) is induced by either high external Ca^{2+} or the addition of the calcium ionophore A23187 in the dark (18). In this species, there is K^+ channel activation by either high external Ca^{2+} or A23187 (15). However, systrophe is blue light-induced, whereas K^+ channels are activated by a transition to dark. Therefore, obligatory coupling of the two processes is unlikely, although they appear to share in part the same transduction pathway.

In summary, the measurement of the action spectrum for K^+ channel activation made it possible to confirm that activation was via phytochrome, and to show that, like some other reported phytochrome-mediated processes, K^+ channel activity is partially activated by shorter wavelength light. Photosynthesis does not activate the K^+ channel. Partial activation by blue light suggests that the transduction pathway for both K^+ channel activation and chloroplast rotation may share two primary photoreceptors. We cannot infer that K^+ channel activation is part of the process of chloroplast rotation. The K^+ channel may function in voltage clamping the membrane to the Nernst potential for K^+, or even in allowing movement of Ca^{2+} into the cytoplasm (8). Certainly there are a number of cases in which phytochrome has been shown to affect the membrane potential of higher plant cells and algae (6). Typically, phytochrome activation causes depolarization. If K^+ channels can be implicated, they may play a role in maintaining a negative-inside potential during the process of transduction.

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