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Activation of K^+ Channels in the Plasma Membrane of Arabidopsis by ATP Produced Photosynthetically

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Light activates a K^+ channel and transiently depolarizes the plasma membrane of Arabidopsis mesophyll cells. Genetically or chemically impairing photosynthesis abolished this electrical response to light. These results indicate that illuminated chloroplasts produce a factor that activated K^+ channels in the plasma membrane. By patch clamping at the single-channel level, we have obtained evidence that ATP is one such factor. Application of 0.2 to 2 mM ATP to the cytoplasmic side of excised patches of membrane reversibly activated the type of channel that was activated by light in cell-attached patches. In addition, an outward-rectifying K^+ channel and an outward-rectifying nonselective cation channel were similarly activated by ATP, whereas a nonselective stretch-activated channel was unaffected by this treatment. This novel mechanism for controlling the permeability of the plasma membrane to K^+ may be important to photosynthetic metabolism.

INTRODUCTION

Ion channels are integral membrane proteins that mediate the movement of ions down their electrochemical-potential gradients. The fraction of time a channel spends in an open, transporting state (open probability [P_o]) controls its contribution to the macroscopic membrane currents that are central to many cellular processes such as signal transduction, osmoregulation, and differentiation (Jaffe, 1981; Schroeder and Hedrich, 1989; Tester, 1990). The opening and closing of an individual channel molecule residing in a small patch of membrane can be monitored with the patch–clamp technique. Determining factors that influence the P_o of specific channels provides clues as to how membrane permeability and, thus, transmembrane currents are controlled.

Permeability to K^+ mediated by channels is ubiquitous among the plasma membranes of eukaryotes (Hille, 1992) and serves many diverse functions. In plants, the resulting K^+ flux repolarizes the membrane during an action potential (Mummert and Gradmann, 1991) and contributes to the changes in turgor pressure underlying the movements of pulvinar motor cells (Moran et al., 1988) and stomatal guard cells (Blatt, 1991). We have been employing the patch–clamp technique to investigate the ion channels that confer K^+ permeability to the plasma membrane of mesophyll cells, both in oat (Kourie and Goldsmith, 1992) and Arabidopsis, where three types of channels that transport K^+ can be distinguished on the basis of their distinctly different current–voltage (I_o-V_m) relations (Spalding et al., 1992). Two of these channel types were shown to be selective for K^+ over Na^+ and Cl^- and were assigned the acronyms PKC1 and PKC2. The third type is a cation channel named PCC1 that transports K^+ and Na^+ but not Cl^-. At least one of these types of channels (PKC1) is activated by light (Spalding et al., 1992).

Activation of K^+ channels by light in a photosynthetic cell suggests the channels may be sensitive to some aspect of metabolism. In mammalian cells, there is a class of K^+ channels whose activity is coupled to metabolism by ATP (Noma, 1983; Weiss and Lamp, 1987; Rorsman and Trube, 1990). The recent demonstration of some similarity in the sequences of genes encoding plant and animal K^+ channels (Anderson et al., 1992; Schachtman et al., 1992; Sentenac et al., 1992) highlights the possibility that metabolic control has also been conserved. The results presented here address this possibility while providing an explanation for the effect of light on K^+ channels in Arabidopsis.

RESULTS

Photosynthesis Affects Membrane Potential

White light delivered to an intact wild-type Arabidopsis leaf induced a depolarization of the membrane that was followed by oscillations in membrane potential (V_m), as shown in the top trace of Figure 1. Light-off was followed by a transient hyperpolarization. Treatment of a wild-type leaf with DCMU, which inhibits photosynthetic electron transport, abolished these light-related changes in V_m (middle trace, Figure 1). The bottom trace in Figure 1 shows that a leaf of the albino mutant *alb-1*,

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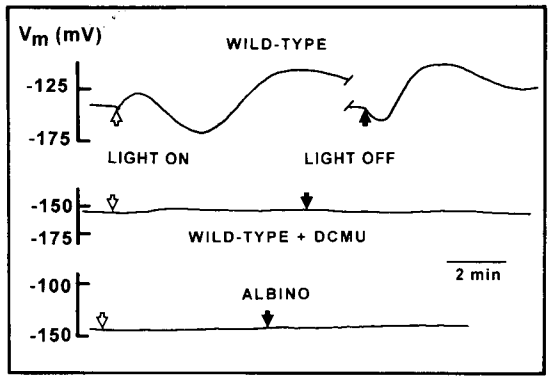

Figure 1. Light Acting through Photosynthesis Changes Membrane Potential of Mesophyll Cells.

White light (400 μmol m^{-2} sec^{-1}) induces a transient membrane depolarization in intact wild-type Arabidopsis leaves (top trace). A transient hyperpolarization follows light-off. Responses to light-on (open arrows) and light-off (closed arrows) are nearly abolished by DCMU (middle trace) and are not detectable in the albino mutant *alb-1* (bottom trace).

which is unable to perform photosynthesis, did not display these electrical responses to light. Albinism in this mutant results from a recessive mutation on chromosome 1 (Koornneef et al., 1983). Wild-type plants cocultured with the mutant (see Methods) displayed a wild-type response to light (data not shown). Results similar to those above were obtained in at least three independent experiments.

ATP Activates the K^+ Channel PKC1

The recording of channel currents from an excised, inside-out patch shown in Figure 2 demonstrates that two individual channels of the same type were activated by ATP applied to the cytoplasmic side of the patch. With V_m clamped at 0 mV, the open channel allows outward K^+ current, which is displayed as an upward shift from the baseline. Switching the solution at the cytoplasmic side of the patch from 0.2 to 2 mM ATP increased P_o (the proportion of time the channels were open), and returning ATP to 0.2 mM reversed this stimulation. Stepping V_m to −50 and −80 mV yielded open-channel current (I_o) data for the channels in this patch. When I_o is plotted versus V_m, as shown in Figure 3 (open symbols), the points lie close to the I_o-V_m curve (solid line) for the type of K^+ channel designated PKC1 that was published previously (Spalding et al., 1992). Dependence of PKC1 activity on ATP in the 0.2 to 2 mM range was observed in three independent trials and at membrane voltages both positive and negative to the K^+ equilibrium voltage (E_K).

The data in Figure 4 demonstrate that ATP approximately doubled the activity ($N \cdot P_o$, see Methods) of at least three channels in a patch in a reversible fashion. The channel currents displayed are representative of the data included in the histogram bin that bears the same letter. Channel openings are plotted downward because the resulting K^+ currents are inward (negative) at V_m = −100 mV. Inspection of such recordings suggests that ATP reduced the time the channels spend in a closed state, as opposed to an increase in their open times. The I_o-V_m data shown in Figure 3 (closed symbols) were obtained from the channels in this patch and are indicative of PKC1. This method of comparing I_o-V_m curves provides a reliable means of identifying the distinctly different channels in Arabidopsis, provided the ionic conditions are similar to those used in the initial characterization.

ATP Activates the Nonselective Cation Channel PCC1

The continuous recording in Figure 5 demonstrates that ATP activated the nonselective cation channel designated PCC1 (Spalding et al., 1992). The irregular amplitudes of the openings (upward shifts from the baseline at V_m = 0 mV) indicate that two or more types of channels having different conductances were active in this patch. A 2 mM ATP solution at the cytoplasmic side of the patch was replaced by an ATP-free solution, and then was switched back to 2 mM ATP where indicated. $N \cdot P_o$ is clearly dependent upon ATP. The I_o-V_m curve obtained for the predominant channel in this patch is displayed in Figure 6. The curve fitted by eye to the data is

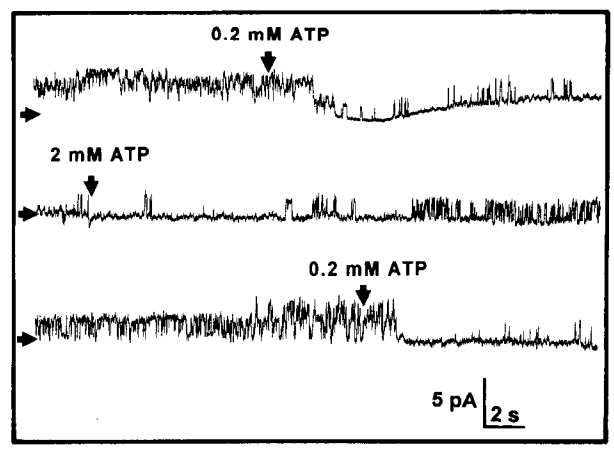

Figure 2. K^+ Channels Activated by ATP in an Excised Patch of Plasma Membrane.

Channel currents were recorded from an inside-out patch clamped at 0 mV. A switch from 2 to 0.2 mM ATP was made at the first vertical arrow, from 0.2 to 2 mM ATP at the second vertical arrow, and back to 0.2 mM ATP at the third vertical arrow. In addition to ATP, the bathing solution contained 220 mM KCl, 4 mM Na^+, 1 mM Ca^{2+} (see Methods) buffered to pH 7.0, and the pipette contained 50 mM KCl, 1 mM $CaCl_2$. Horizontal arrows to the left of the traces indicate the closed-state current level. Filter frequency was 40 Hz.

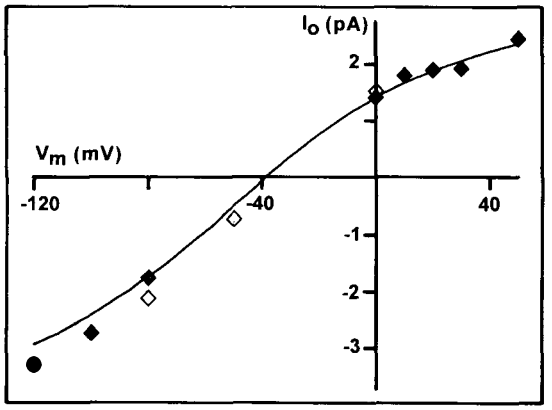

Figure 3. Identification of the Channels Activated by ATP in Figure 2.

The I_o-V_m curve for PKC1-type K^+ channels (Spalding et al., 1992) is redrawn here. ◇, data from channels in Figure 2; ♦, data from channels in Figure 4. E_K = −38 mV and E_{Cl} = +38 mV.

characteristic of PCC1 and intercepts the voltage axis very near E_K = −38 mV. Channel currents at the upper left of Figure 6 bear a letter that corresponds to a point on the I_o-V_m curve. Activation of PCC1 by ATP was observed in two patches.

ATP Activates the Outward-Rectifying K^+ Channel PKC2

ATP also activated the outward-rectifying K^+-selective channel PKC2, as shown in Figure 7. $N \cdot P_o$ increased more than fivefold upon the addition of 2 mM ATP and decreased following its removal. V_m was clamped at 20 mV during the addition and removal of ATP, but data were also obtained at other voltages to construct the I_o-V_m curve (upper right corner) that identifies these channels as PKC2. The I_o-V_m curves for PKC2 and PCC1 are qualitatively similar (Spalding et al., 1992), but I_o = 3.3 pA at V_m = 40 mV for PKC2 (Figure 7) and 5.0 pA for PCC1 (Figure 6) at the same voltage. This quantitative difference readily separates them. PKC1, on the other hand, displays a relatively flat I_o-V_m curve at positive voltages and I_o = 2.2 pA at V_m = 40 mV. Activation of PKC2 by ATP was observed in three patches.

Stretch-Activated Channel Is Unaffected by ATP

Figure 8 displays a recording of current through stretch-activated ion channels (at least two in the patch). The I_o-V_m curves were obtained from separate patches and with different bathing solutions. This type of channel, previously undescribed, opened immediately upon the application of suction to the pipette (~1 to 5 kPa) and remained active while the suction was maintained. This channel has not been observed to open in the absence of suction. Openings in the presence of suction at positive membrane voltages are rare, indicating a strong voltage dependence of P_o. Release of the suction was followed by closure of the channels. The curves in the lower part of Figure 8 were fitted to the I_o-V_m data by eye. With 100 mM KCl at the cytoplasmic side, the current appears to reverse very near 0 mV. This indicates that the channel was equally permeable to K^+ and Cl^-. Linear extrapolation of the last several data points obtained with 220 mM KCl yielded V_m = 10 mV as the reversal voltage, indicating a permeability ratio (Cl^- to K^+) of 1.9. Measurements of I_o at additional positive voltages would better define the selectivity of this channel. Such data may be obtained by using a voltage ramping protocol. Until then, the channel will be considered a nonselective stretch-activated ion channel.

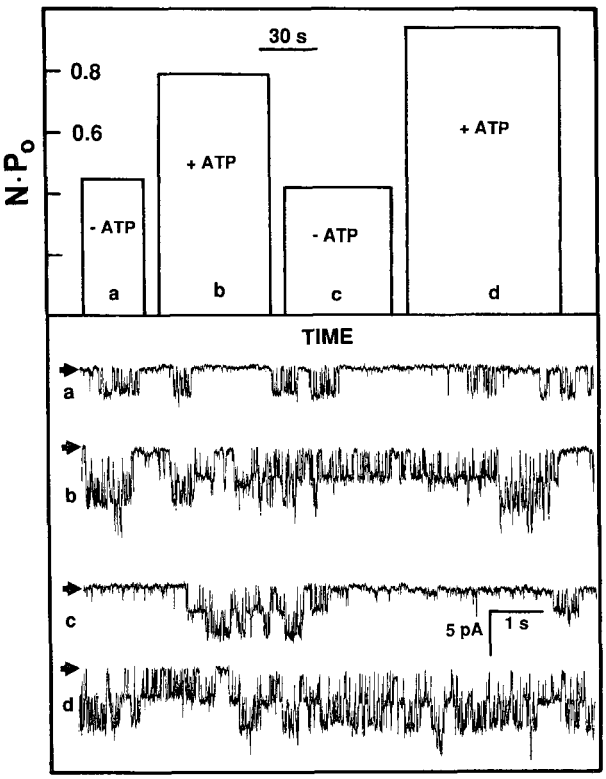

Figure 4. Quantitation of Channel Activity ($N \cdot P_o$) in the Presence and Absense of ATP.

An inside-out patch clamped at V_m = −100 mV was repeatedly exposed to 0 or 2 mM ATP. Solutions were as given in Figure 2. Recordings of single-channel currents (lower part) correspond with the histogram bin bearing the same letter. The arrows to the left of the traces indicate the closed-state current level. The gap between histogram bins represents ~5 sec of data lost due to electrical noise introduced by opening the Faraday cage to switch solutions. Filter frequency was 500 Hz.

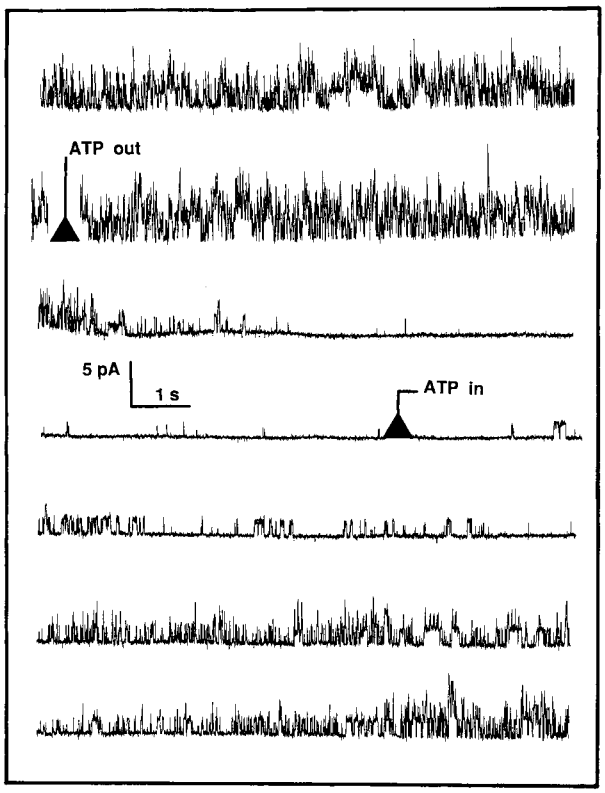

Figure 5. ATP Activation of a Nonselective Cation Channel.

Continuous recording of current across an inside-out patch with 2 mM ATP initially in the bath. The activity of the channels in the patch (more than one type present) decreases upon removal of the ATP and increases upon its reapplication. Approximately 5 sec of data was lost at each switch, as explained in Figure 4. Solutions are as given in Figure 2. Filter frequency was 500 Hz.

The stretch-activated channel was unaffected by ATP. Figure 9 displays currents through at least two of these channels in a patch clamped at V_m = −120 mV with 3.5 kPa of suction applied continuously. $N \cdot P_o$ was unaffected by switching between 0.2 and 2 mM ATP (Figure 9) or between 0 and 2 mM ATP (data not shown, n = 2).

DISCUSSION

We demonstrated previously that white light activates a K^+ channel (PKC1) in cell-attached patches but not in excised (cell-free) patches (Spalding et al., 1992). Provided that V_m is initially more negative than E_K, activation of PKC1 would depolarize the membrane as was observed (Figure 1), although changes in other conductances likely contribute to the full response. When combined with the evidence that photosynthesis mediates the light-induced changes in V_m (Figure 1), the above information indicates that illuminated chloroplasts produce a factor that diffuses to the plasma membrane and activates K^+ channels. A role for chloroplasts in mediating light-induced changes in ion transport at the plasma membrane has been indicated by other studies (Brinkmann and Lüttge, 1974; Tazawa and Shimmen, 1980; Serrano et al., 1988; Vanselow and Hansen, 1989), but factors responsible for the coupling were not identified.

We propose that ATP generated by photosynthesis activates K^+ channels in the plasma membrane of Arabidopsis. The evidence supporting this is that light delivered to cell-attached patches activates the same type of channel (Spalding et al., 1992) as ATP applied to inside-out patches (Figures 2 to 4). Furthermore, cytosolic ATP increases transiently upon illumination (Santarius and Heber, 1965; Hampp et al., 1982; Laisk et al., 1991), probably through the operation of the 3-phosphoglyceric acid/dihydroxyacetone phosphate shuttle rather than by direct export from the chloroplast via the adenine nucleotide translocator (Goodwin and Mercer, 1983).

The results reported here (Figure 2) show that ATP concentrations falling within the range of cytosolic concentrations were

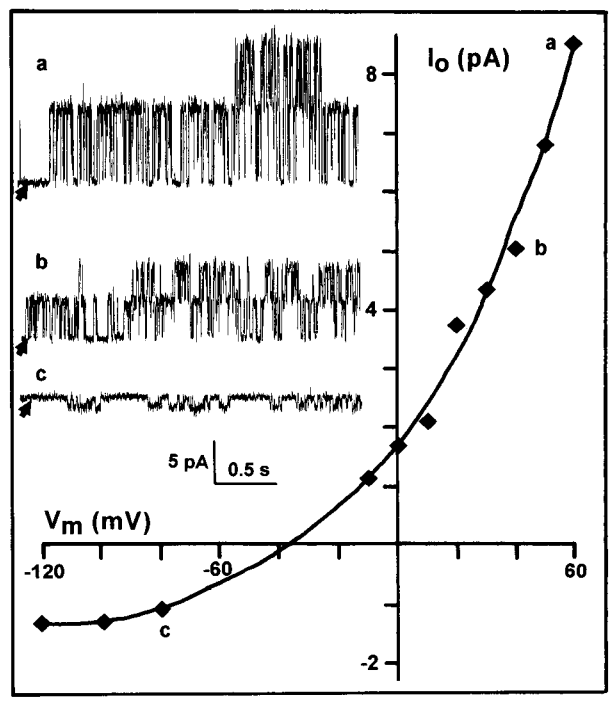

Figure 6. Identification of the ATP-Activated Channel in Figure 5.

The I_o-V_m curve of the predominant channel in Figure 5 is characteristic of PCC1, a nonselective cation channel. The displayed channel currents (upper left) were recorded from the same patch and correspond with the point on the curve bearing the same letter. Arrows to the left of the traces point to the closed-state current level. Filter frequency was 500 Hz.

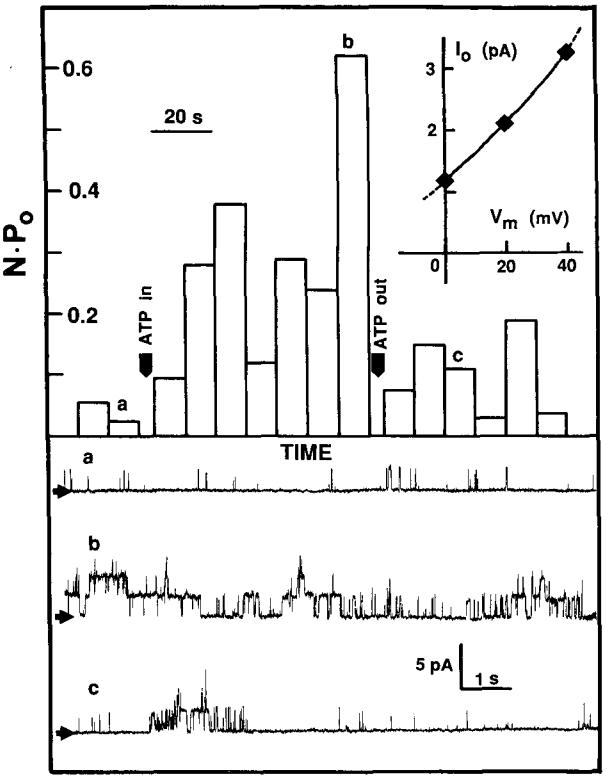

Figure 7. ATP Activation of the Outward-Rectifying K^+ Channel PKC2.

$N \cdot P_o$ of at least three channels in the patch is presented in sequential 10-sec bins. The channel currents displayed below correspond with the bin bearing the same letter. The I_o-V_m data in the upper right are from the same patch and identify the channels as PKC2. The solutions are as given in Figure 2. Filter frequency was 500 Hz.

effective in activating this channel and indicate that this control mechanism is physiologically important. Further investigations of this novel regulatory mechanism will determine the specificity of the activation for different nucleotides by comparing the concentration of each required to elicit half-maximal activation. In addition to possibly competing with ATP, GTP may influence the channel by a different control mechanism involving G-proteins (Fairley-Grenot and Assmann, 1991). Furthermore, a nonspecific nucleotide receptor may mediate the activation of whole-cell anion currents in guard cells by 0.1 mM ATP and GTP (Hedrich et al., 1990).

In mammalian cells, ATP reduces the activity of a type of K^+ channel known as K_{ATP} (Noma, 1983; Rorsman and Trube, 1990). The effect at the cellular level is a coupling of K^+ permeability to metabolism, which is important, for different reasons, in the contraction of muscle cells (Spruce et al., 1985), glucose-induced secretion in pancreatic B cells (Rorsman and Trube, 1990), and in the coordination of channel and pump activities in renal epithelial cells (Tsuchiya et al., 1992). ATP reversibly binds to K_{ATP} channels and reduces P_o without being hydrolyzed.

The K^+ permeability of the plasma membrane in Arabidopsis mesophyll cells is oppositely coupled to cellular metabolism because ATP increases the activity of K^+ channels. This may be important to photosynthetic activity in mesophyll cells. A K^+ current from the cytoplasm into the chloroplast stroma is required to balance the efflux of H^+ from the stroma that is observed upon illumination (Wu and Berkowitz, 1992). The alkalinization of the stroma increases the rate of photosynthesis by increasing the activities of enzymes of the Calvin cycle (Heldt, 1979). Therefore, a light-induced increase in the K^+ permeability of the plasma membrane, achieved via ATP-activated channels, may facilitate the proper control of stromal

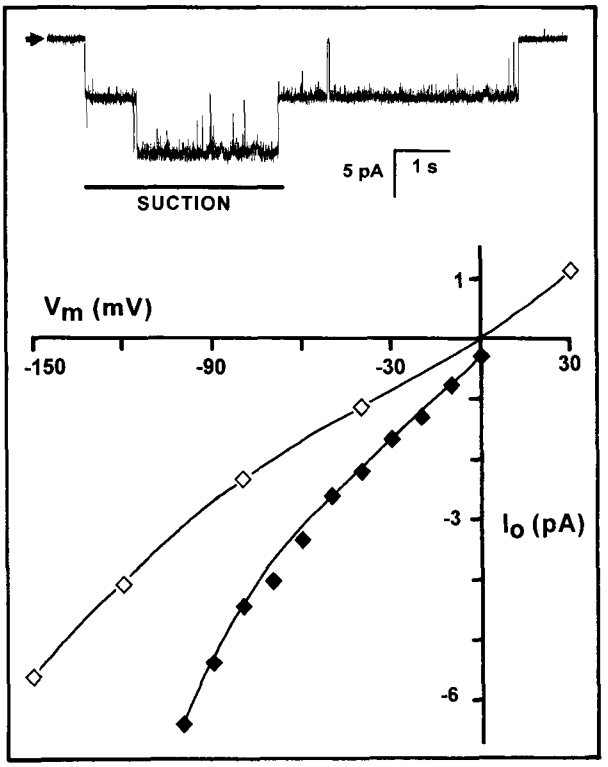

Figure 8. Stretch Activation of a Nonselective Channel.

In the top section, suction was applied to the pipette for the duration indicated by the horizontal bar. Two channels in an inside-out patch clamped at V_m = −100 mV opened upon the onset of suction and closed following its release. The horizontal arrow indicates the closed-state current level. The bath contained 220 mM KCl and 0.1 mM Ca^{2+}. Filter frequency was 300 Hz. In the lower section, I_o-V_m data demonstrate nonselectivity of this channel. ♦, data obtained with 220 mM KCl in the bath so that E_K = −38 mV and E_{Cl} = +38 mV. ◇, data obtained with 100 mM KCl and 150 mM sorbitol in the bath so that E_K = −18 mV and E_{Cl} = +18 mV. Ca^{2+} was buffered to 0.1 mM with 5 mM EGTA in both bathing solutions.

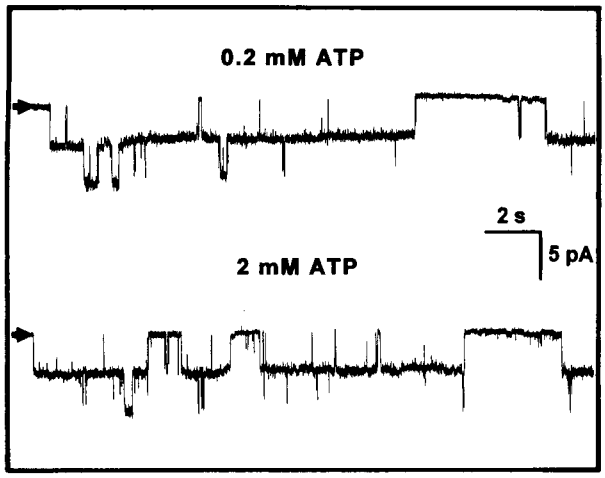

Figure 9. Stretch-Activated Channel Not Affected by ATP.

A patch containing at least two stretch-activated channels was subjected to a constant suction of 3.5 kPa. Switching between 0.2 and 2 mM ATP did not affect P_o. V_m was clamped at −120 mV. The bath contained 100 mM KCl and 150 mM sorbitol. Ca^{2+} was buffered to 0.1 mM with 5 mM EGTA. Filter frequency was 300 Hz.

pH by increasing the cytosolic concentration of K^+. It remains to be determined whether kinase and phosphatase activities associated with the channel in the patch of membrane mediate the effects of ATP addition and removal, or whether ATP simply binds to the channel and modulates its activity without being hydrolyzed.

A light-induced change in cytosolic Ca^{2+} was also suggested to coordinate photosynthetic metabolism in Nitellopsis (Miller and Sanders, 1987). However, directly extending observations from Nitellopsis to Arabidopsis may not be useful because ATP has been reported to inhibit a K^+ channel in this alga (Katsuhara et al., 1990). Nonetheless, the results of Miller and Sanders (1987) emphasize the need to consider the involvement of other factors, such as Ca^{2+} (Schroeder and Hagiwara, 1989; Stoeckel and Takeda, 1989; Ketchum and Poole, 1991), H^+ (Steigner et al., 1988), and inorganic phosphate (Takeshige et al., 1992) in the control of light-induced ion fluxes. Furthermore, photosynthetically generated changes in the redox potential of the cytoplasm (Heineke et al., 1991) may also play a role, because the Arabidopsis channels are activated by artificial reducing agents applied to the cytoplasmic side of the membrane (Spalding at al., 1992).

It appears that light of sufficient irradiance can cause V_m to shift to voltages more positive than E_K (Spalding et al., 1992), possibly by activating channels selective for Cl^- or Ca^{2+} in addition to PKC1. In this case, currents through the outward-rectifying channels PKC2 (Figure 7) and PCC1 (Figure 6) would tend to repolarize the membrane toward E_K. Although it is not yet known if these channels are activated by light in cell-attached patches, their activation by ATP (Figures 5 and 7) indicates they may be. Increasing the permeability of the membrane to K^+ by increasing cytosolic ATP would be expected to dampen changes in V_m caused by changes in other conductances and buffer V_m closer to E_K. Repolarization of the membrane to values more negative than E_K could be achieved if the channel activation is transient or if light activated the H^+-ATPase (Spanswick, 1982; Serrano et al., 1988).

Activation by ATP is not an entirely general feature of ion channels in Arabidopsis because ATP does not affect the stretch-activated channel (Figure 9). This channel is nonselective and displays long open times and a high conductance. The stretch-activated channels found in fungi (Gustin et al., 1988; Zhou et al., 1991; Zhou and Kung, 1992), bacteria (Martinac et al., 1992), and cultured tobacco cells (Falke et al., 1988) share combinations of these characteristics, but those in the guard cells of broad bean do not (Cosgrove and Hedrich, 1991). Stretch-activated channels are thought to be involved in the regulation of turgor pressure either directly, by allowing the efflux of osmotically active solutes when tension in the membrane exceeds a certain threshold, or indirectly, by transducing membrane tension into a signal interpretable by a separate osmoregulatory mechanism. The lack of sensitivity of the stretch-activated channel to ATP further indicates that the water relations of the cell are more relevant to its control than cellular metabolism.

METHODS

Plant Material

Wild-type *Arabidopsis thaliana* (Landsberg genotype) plants were grown in pots as previously described (Spalding et al., 1992). The plants were illuminated with white light for 16 hr each day at a fluence rate of 110 μmol m^{-2} sec^{-1}. The albino mutant *alb-1* was grown by sowing a segregating population of surface-sterilized seeds in sterile Petri plates containing full-strength Murashige-Skoog salts (Murashige and Skoog, 1962) plus 3% sucrose and 1% agar. Approximately 25% of the plants were albino (homozygous recessive). The remainder were green, and both were smaller than pot-grown plants of similar age. However, the leaves of green plants grown in pots and in culture displayed similar light-induced changes in membrane potential (V_m). The protoplasts for patch clamping were prepared from pot-grown wild-type leaves as previously described (Spalding et al., 1992), except that the sorbitol concentration in the protoplast isolation medium was lowered from 0.6 to 0.5 M.

Electrophysiology

The electronics, patch-clamping procedures, and notation conventions have been previously described (Spalding et al., 1992). Minor changes were made in the mounting of intact leaves for measuring V_m. Excised leaves were placed abaxial-side down in a drop of slightly warm 1% agar containing 1 mM $CaCl_2$ and 1 mM KCl in the bottom

of a plastic Petri dish (35-mm-diameter). After the agar solidified, a solution containing 1 mM $CaCl_2$ and 1 mM KCl with or without 0.1 mM DCMU was added to the dish such that the edges but not the entire upper surface of the leaf were submerged. Leaves of the albino were treated with the same solution supplemented with 3% sucrose. A lid was placed on the dish, and the leaf was allowed to recover for at least 3 hr in the dark. The exposed part of the leaf was impaled with an intracellular microelectrode, and the Ag/AgCl reference electrode contacted the nonflowing bathing solution by way of a 1 M KCl–1% agar salt bridge. Light was applied after V_m had been stable for at least 2 min. Changes in V_m such as those induced by light-on and light-off were never observed in the absence of those treatments.

Solutions and Treatments

In every patch-clamp experiment, the pipette contained 50 mM KCl, 1 mM $CaCl_2$, 5 mM Hepes, adjusted to pH 7.0 with 1,3-bis(tris-(hydroxymethyl)methylamino)propane. The composition of the bathing solution (cytoplasmic side) is given in the figure legends. Differences in the concentration of Na^+, introduced as the disodium salt of ATP, were balanced by adding the appropriate amount of NaCl. In Figure 2, a constant, unchelated Ca^{2+} concentration of 1 mM was achieved by adding 2.39 mM $CaCl_2$ to the bathing solutions containing 2 mM ATP, and 1.14 mM $CaCl_2$ was added to those containing 0.2 mM ATP. In the other experiments, no compensation for the Ca^{2+}-chelating effect of ATP was made. All bathing solutions were buffered to pH 7.0 with 5 mM Hepes and 1,3-bis(tris(hydroxymethyl)methylamino)propane after the addition of ATP.

The light source used to induce changes in V_m was the same as previously described (Spalding et al., 1992), but the fluence rate was lowered to 400 μmol m^{-2} sec^{-1}.

Suction was applied by mouth and quantitated by measuring the vertical displacement of a water column in a U-shaped glass tube connected in line with the pipette. A given suction could be maintained by turning a stopcock to close the system.

Quantitation of Channel Activity

The channel currents were digitized by a computer at a rate at least double the filter frequency stated in the figure legends as previously described (Spalding et al., 1992). Gaussian distributions were fitted to all-point histograms constructed from the digitized recordings using pCLAMP software (Axon Instruments, Foster City, CA). The difference between the closed- and open-state peaks was taken as I_o. When more than one channel of the same type was active in a patch, activity ($N \cdot P_o$) was calculated with the formula

$$N \cdot P_o = \frac{C_1 + 2C_2 + \ldots + nC_n}{\text{total counts}}$$

where C_1 is the number of counts comprising the peak corresponding to one open channel; C_2 is the number of counts comprising the peak corresponding to two channels open simultaneously; C_n is the number of counts comprising the peak corresponding to n channels open simultaneously, and n is the maximum number of simultaneously open channels observed.

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