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ORIGINAL RESEARCH

Regulation of Dark-Induced Stomatal Closure in *Arabidopsis* Dynamin-Like Protein 1E (*adl1e*) Mutant Leaves

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Abstract *Arabidopsis thaliana* dynamin-like protein 1E (ADL1E) is known to regulate mitochondrial elongation. The *adl1e* mutant has no morphological phenotype, and the growth and photosynthetic activity of the mutant are similar to those of the wild type. Leaf O_2 uptake, which is supported by mitochondrial activity in the dark, is increased 1.7-fold by mutation in *adl1e* gene. The ATP content in the dark of guard and mesophyll cell protoplasts (GCPs and MCPs, respectively) was 2.5- to 4-fold higher in GCPs of the mutant and the wild type, and increased upon the addition of glucose in both genotypes. Oligomycin, an inhibitor of mitochondrial ATPase, suppressed ATP synthesis in both GCPs and MCPS isolated from *adl1e* plants, indicating that mutant had higher mitochondrial activity. The stomatal apertures of mutant and wild-type plants were then analyzed in vitro. In the light, the stomata of both genotypes showed similar patterns of opening. However, in the dark response, the stomata of the *adl1e* mutant closed faster than did those of the wild type. Oligomycin severely inhibited dark-induced stomatal closure in both cell types. The results suggest that stomatal closure in the dark is governed by cytosolic ATP concentration, which is stimulated by mitochondrial activity.

Keywords ADL1E · *Arabidopsis* · ATP · Dark · Mitochondria · Stomata

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Introduction

In the epidermal tissue of most plant species, only guard cells contain chloroplasts, and these have been suggested to play an important role in stomatal opening. Blue light (BL) is most effective in inducing stomatal opening in a background of red light (RL), which is absorbed by guard cell chloroplasts (GCCs) (Ogawa et al. 1978; Assmann et al. 1985; Iino et al. 1985; Shimazaki et al. 1986). GCCs supply ATP to the cytosol in the presence of RL, and this ATP is used by the plasma membrane H^+-ATPase for H^+ pumping (Tominaga et al. 2001). In response to stimuli that elicit stomatal opening, K^+ influx increases the osmotic pressure of guard cells, which results in water uptake, a bowing out of the two guard cells that define each stomatal pore, and an increase in stomatal aperture. K^+ influx occurs through inwardly rectifying K^+ channels and is driven by membrane hyperpolarization caused by H^+ ATPase activation and anion channel inactivation. Conversely, stimuli that elicit stomatal closure inhibit H^+ ATPase activity and activate anion channels, which results in membrane depolarization that both

activates outwardly rectifying K^+ channels and provides the driving force for K^+ efflux (Assmann 1993; Ward et al. 1995) These ion channels that facilitate stomatal closure are regulated by abscisic acid (ABA) in the presence of cytosolic ATP (Schmidt et al. 1995; Schwarz and Schroeder 1998). As well, a continuous supply of ATP is also required for stomatal closing in response to darkness (Karlsson and Schwartz 1988; Cousson et al. 1995). However, this views that cytosolic ATP requirement for dark-induced stomatal closure is much relied on pharmacological studies but lacked molecular genetic approaches.

Dynamin-related proteins (DRPs) are large GTPases that occur in a variety of eukaryotic organisms, from yeast to human (Konopka and Bednarek 2008), and deform and cause fission of membranes (Praefcke et al. 2004). Based on their amino acid sequences and functional domains, they are classified into sub-families DRP1 to 6, which have been linked to cell plate formation, endocytosis, exocytosis, protein sorting, and mitochondrial and chloroplast division (Hong et al. 2003a, b). Functional classification recognizes two groups of DRPs, namely tubulases, which draw membranes out into long tubules, and pinchases, which release vesicles from constricted membranes (Verma and Hong 2005).

Plants contain more dynamin homologs than do mammals, and there is therefore expected to be some functional redundancy among the plant DRPs. For example, there are at least five isoforms (A–E) of the *Arabidopsis thaliana* dynamin-like protein 1 (ADL1; Praefcke et al. 2004). Among these, ADL1E is thought to play a critical role in mitochondrial fission in plant cells, as a T-DNA insertion mutant of *adl1e* had abnormally elongated mitochondria and ~40% fewer mitochondria per cell (Jin et al. 2003).

The absence of DRP1E did not show any visible phenotypic changes (Kang et al. 2003) but exhibited mitochondria in abnormal shape with about 61% increase in length (Jin et al. 2003). However, it is not known whether aberrant mitochondria are functionally related to stomata movement in *adl1e* mutant leaves. In an attempt to elucidate the ATP-dependent mechanism that underlies stomatal closure in darkness, we studied the effect of oligomycin, an inhibitor of mitochondrial ATPase, on dark-induced stomatal closure in *adl1e* mutant leaves. Our data suggest that cytosolic ATP is indeed required for dark-induced stomatal closure and that mitochondrial activity is therefore important in this process.

Materials and Methods

Plant Growth

Wild-type and transgenic *A. thaliana* (ecotype Columbia) plants were grown on soil in a greenhouse at an irradiance of 110 μmol m^{-2} s^{-1} and at a relative humidity of 70%. The cultures were maintained in an environmentally controlled growth room at 21±2°C on a 16:8-h light/dark cycle. For growth analysis, the plants were grown on agar plates with 0.5×B5 medium (pH 5.7) in petri dishes.

Chlorophyll *a* Fluorescence Measurements

Analysis of fluorescence quenching parameters during illumination was conducted with a Xe-PAM fluorometer (Heinz Walz, Effeltrich, Germany) as previously described (Choi et al. 2002). The effective quantum yield of PS II was defined as $\Delta F/F_m' = (F_m' - F)/F_m'$ (Genty et al. 1989).

Determination of Net O_2 Evolution and Net O_2 Uptake

Rates of net O_2 evolution and net O_2 uptake were determined at room temperature on detached leaves using an Oxygraph system (Hansatech, King's Lynn, Norfolk, UK) in a closed gas exchange system, which included air with 5% CO_2 (Goh et al. 2004). The system was calibrated according to the method of Walker (1987). The actinic light was provided by a 150-W quartz-halogen slide projector and passed through neutral density filters.

Isolation of Guard and Mesophyll Cell Protoplasts

Guard cell protoplasts (GCPs) were isolated as previously described (Goh et al. 1999). The epidermal peels were rinsed first with deionized water and then subjected to a two-step enzymatic digestion, with slight modification. Briefly, the peels were transferred into a 250-ml flask containing 50 ml of basic solution with 1.5% (*w*/*v*) cellulase Onozuka RS (Yakult Pharmaceutical Co., Tokyo, Japan), 250 mM sorbitol, 0.5% (*w*/*v*) bovine serum albumin (BSA), 1 mM $CaCl_2$, and 10 mM Na-ascorbate (pH 5.5) for 1 h at 22°C. After incubation, the peels were gathered on a Nylon net (200 μm mesh size) and thoroughly washed in 400 mM sorbitol containing 1 mM $CaCl_2$. The peels were then subjected to a second digestion for 3.5 h at 22°C, using medium containing 1.5% (*w*/*v*) cellulase Onozuka RS, 0.05% (*w*/*v*) pectolyase Y-23 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), 400 mM sorbitol, 0.5% (*w*/*v*) BSA, 1 mM $CaCl_2$, and 10 mM Na-ascorbate (pH 5.5). The released protoplasts were harvested by filtration through a Nylon net (10 μm mesh size), collected by centrifugation (200×*g*; 5 min), and rinsed in 400 mM sorbitol containing 1 mM $CaCl_2$. The GCPs are then further processed for purification by Histopaque (No. 1077, Sigma Chemical Co.) as described in the method of Pandey et al. (2001). The typical purity is 99.2–99.6% on a cell basis, with contamination originating from mesophyll cells (data not shown). Isolated protoplasts were suspended in 400 mM sorbitol

containing 1 mM $CaCl_2$ and kept in the dark on ice until used.

For comparative measurements, mesophyll cell protoplasts (MCPs) were also isolated from the leaves. The procedure was performed as described previously (Goh et al. 1999), with slight modifications. The enzymatic solution used here consisted of 0.5% (*w*/*v*) macerozyme R-10 (Yakult Pharmaceutical Co.), 1% (*w*/*v*) cellulase Onozuka RS, 0.5% (*w*/*v*) BSA, 600 mM sorbitol, and 1 mM $CaCl_2$ (pH 5.5). The incubation was carried out for 1 h at 22°C. Isolated protoplasts were suspended in 600 mM sorbitol containing 1 mM $CaCl_2$ and kept in the dark on ice until used.

ATP Measurements

The amount of ATP in isolated protoplasts was measured using the luciferine–luciferase method (Manfredi et al. 2002). The cells were incubated in a temperature-controlled room (21°C) for 1 h in the dark. The incubation medium was composed of 0.4 M (for GCPs) or 0.6 M sorbitol (for MCPs), 1 mM $CaCl_2$, 10 mM KCl, 10 mM MES-NaOH (pH 6.2), and protoplasts (30 μg/ml) and contained different combinations of substrate and inhibitors. ATP was extracted from 100 μl of cell suspension using 2.5% trichloroacetic acid (TCA) and an ENLITEN ATP Assay System Bioluminescence Detection Kit (Promega), according to the manufacturer's instructions. The extracted ATP solution was neutralized with 0.75 M Tris-acetate buffer (pH 7.75). After centrifugation (1,600×*g*; 10 min), the supernatant was used to quantify the ATP concentration. The reaction was started by adding 100 μl of the extract to 100 μl of the luciferine–luciferase luminous reagent (Promega Co, Madison, WI, USA). The luminescence was integrated for 5 s using a luminometer (Luminoskan Ascent 2.1 Int., Germany).

Measurement of Stomatal Aperture

Stomatal apertures were measured on epidermal strips using bright-field microscopy (Goh et al. 2009). Epidermal strips were prepared from the abaxial surface of rosette leaves that were taken from 8-week-old plants. The epidermal strips were placed in the dark for 2 h and then illuminated with white light (115 μmol m^{-2} s^{-1}) for 3 h. The strips were incubated in 10 mM MES-KOH and 50 mM KCl (pH 6.1) at 22°C. After the illumination, the strips were continuously incubated in the same medium (10 mM MES-KOH and 50 mM KCl; pH 6.1) in the dark for another 6 h. Stomatal apertures were photographed within 3 min of the end of treatment using an Infinity 1 camera coupled to a microscope (www.Imagepro.co.kr) at a 10×20 magnification, and the measurements were then compared with those obtained using an eyepiece graticule that was calibrated with a 100×10-mm slide micrometer scale. Analysis was performed using the I'MEASURE program (v2.0) (INGPLUS Co., Ltd., Canada). Live cells were selected using fluorescein diacetate (Goh et al. 2009) approximately 30 s before the stomatal apertures were measured.

Protein Determination

Protein was determined according to the Bradford (1976) method. BSA was used as the control.

Chlorophyll Content

Chl content was calculated from the absorbances at 646.6 and 663.6 nm using the equations of Porra et al. (1989).

Results and Discussion

Plant Growth and Photosynthetic Activities

The growth of the *adl1e* mutant plant was compared to that of the wild type (WT; Fig. 1a). Under the given conditions, the fresh weight of seedlings increased at a similar rate in both WT and *adl1e* mutant plants, reaching a plateau at about 16 days after germination (Fig. 1b). Total chlorophyll content did not significantly differ on a protein basis between the WT (12.54±0.51) and *adl1e* mutant (12.59±0.78). The ratio of chlorophyll a/b was 2.63±0.12 for the WT and 2.56±0.14 for the *adl1e* mutant. The repetitive fluorescence increases induced by each saturation pulse contained the information needed to perform the quenching analysis and to assess the quantum yield of photosystem II (PS II) and the energy status of the chloroplasts (Genty et al. 1989; Goh et al. 1999, 2002). There were no large differences in the effective quantum yield of PS II between the two plants (Fig. 2a), indicating that the functional organization of thylakoids was similar in the chloroplasts of the *adl1e* mutant and wild type.

Respiratory O_2 Uptake and Photosynthetic O_2 Evolution

The rates of net O_2 uptake and evolution are used as a measure of the rates of mitochondrial respiration and photosynthetic electron transport generated by PSII, respectively (Braidot et al. 1999; Philip and John 2002). The leaves were placed in darkness for 9 min and then exposed to actinic light at 60 μmol m^{-2} s^{-1} for 3 min and at 900 μmol m^{-2} s^{-1} for a further 3 min. This was followed by a further 6-min dark period. During the change from a low light (60 μmol m^{-2} s^{-1}) to a high light (900 μmol m^{-2} s^{-1}) intensity, photosynthetic O_2 evolution changed from 127 to 2,300 nmol min^{-1} g^{-1} fresh weight in both WT and *adl1e*

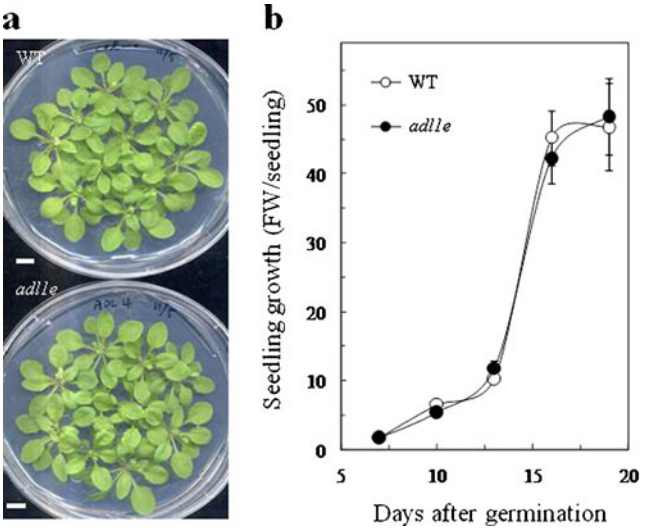

Fig. 1 Plant growth of *adl1e* mutants compared to that of WT plants. **a** Representative photographs of WT and *adl1e* mutant plants at 19 days after germination. **b** Fresh weight (FW) of seedlings measured 7 to 19 days after germination. The values are expressed as mean ± SE (n=40 and n=60 for plants at 19 days after germination). The differences between the means are not statistically significant (t test, P=0.05). *Bars*= 0.78 cm

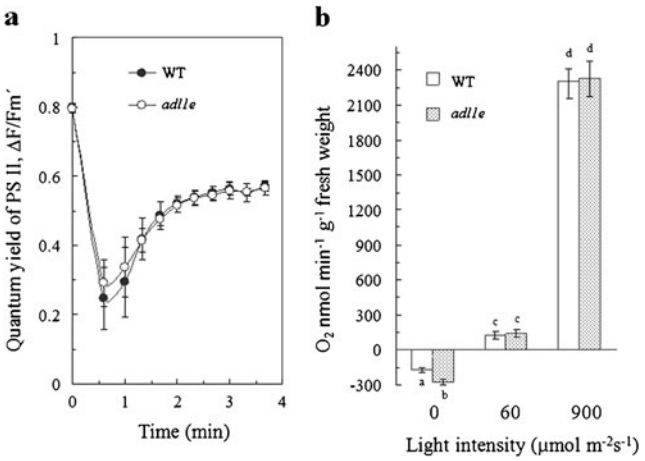

Fig. 2 Photosynthetic activity (**a**) and gross O_2 uptake and evolution (**b**) in leaves of WT and *adl1e* mutant plants. **a** Effective quantum yield of photosystem II. Data are displayed as means ± SE (n=8). The differences between the means are not statistically significant (t test, P =0.05). **b** Gross oxygen uptake and evolution by leaves. Samples were placed in the dark for 9 min prior to data acquisition. Net oxygen exchange rates in the dark (0) or under low (60 μmol m^{-2} s^{-1}) and high (900 μmol m^{-2} s^{-1}) light. For oxygen concentration, + means net oxygen production and − means net oxygen consumption. *Error bars* represent SE of the mean. *Different letters* indicate statistically significant differences between the means (t test, P=0.05). Net oxygen concentration was measured over the last 2-min period of the dark and actinic light intervals. Data were log transformed, such that the gradient of a fitted line, using regression analysis, would constitute the exponential decay constant (k) of the form $R_t=R_0\times e^{-kt}$ (Stone and Ganf 1987), where R_t is the respiration rate at the start of the dark period and R_0 the respiration rate at some time, t

mutant plants (Fig. 2b). However, the rate of oxygen consumption in the dark in *adl1e* mutant plants was 62.1% higher than that of WT plants. This result indicates that the mitochondria of *adl1e* mutant plants have a higher respiratory activity than do those of WT plants.

ATP Content in WT and *adl1e* Mutant Plants

We found that the respiration rates in the dark were significantly higher in the *adl1e* mutant than in the WT (Fig. 3). Since respiration rates have been directly associated with respiratory O_2 uptake and ATP synthesis in mitochondria in the dark (Xie and Chen 1999), we decided to determine the total cellular ATP levels in *adl1e* mutant leaves in the dark. For this, we isolated mesophyll (MCPs) and guard cell protoplasts (GCPs) from both the WT and the *adl1e* mutant. For both MCPs and GCPs, the size of protoplasts did not differ between the WT and *adl1e* mutant (data not shown). ATP content arising from the mitochondria of WT and *adl1e* mutant plants was then measured in MCPs (Fig. 3a) and GCPs (Fig. 3b). Cells were incubated for 1 h in the dark in medium containing or lacking glucose (a stimulant of ATP generation by respiration) or oligomycin (an inhibitor of mitochondrial ATP synthesis). As expected, mitochondrial ATP production was 17.4% greater in MCPs of the *adl1e* mutant than in those of the WT control (−Glucose−Oligo). In the presence of oligomycin, ATP production was significantly inhibited, showing about 89.2% in WT and 85.2% inhibition in *adl1e* mutant cells. The MCPs of both the *adl1e* mutant and the WT had similar in ATP production, and this effect was significantly nullified by the presence of oligomycin in the glucose medium. In GCPs, ATP concentration was 50% greater in the mutant than in the

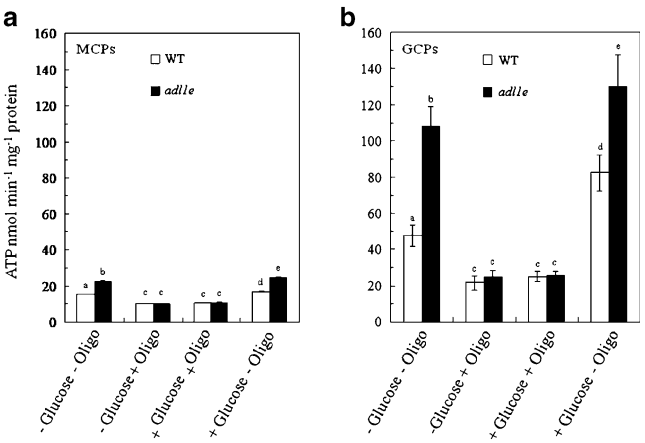

Fig. 3 ATP content in MCPs (**a**) and GCPs (**b**) of WT and *adl1e* mutant leaves. ATP content was measured 3 h after protoplast isolation. Cells were then incubated with different combinations of substrate (Glucose, 2.5 mM) and oligomycin (Oligo, 5 μg ml^{-1}) for 1 h in the dark. *Bars* represent means ± SE (n=5). *Different letters* indicate statistically significant differences between the means (t test, P=0.05) in *each panel*

WT. These respiration activities in both MCPs and GCPs indicate that the mitochondria derived from the *adl1e* mutant cells had a higher rate of oxidative phosphorylation than did those derived from WT cells. Interestingly, mitochondrial activity was about three times greater in GCPs than in MCPs, indicating an enrichment of mitochondrial activity in guard cells relative to that of mesophyll cells as observed previously (Shimazaki et al. 1983; Vani and Raghavendra 1994). We consider that ADL1E plays a role in coordinating the behavior of the inner and outer mitochondrial membranes during fusion, as does Mgm1p (Shaw and Nunnari, 2002). Given that ADL1E is localized to the cristae of the inner mitochondrial membrane (Wong et al. 2000), it might be responsible for further intricate, heavily folded inner membrane of mitochondria, which houses the respiratory chain complexes. Such complexes are responsible for energy production, which is stored as higher ATP (Dudkina et al. 2006). On the other hand, those that perform the redox reactions of oxidative phosphorylation in plant mitochondria are sensitive to light (Pastore et al. 1996; Goh et al. 2010). As a result of irradiation, a decrease in the rate of ATP synthesis was found (Goh et al. 2010). The increased function of the respiratory chain in the *adl1e* mutant under dark is achieved by maintaining the overall ATP production in the leaf cells.

Regulation of Stomatal Closure in Darkness

To assess the effect of the increased mitochondrial activity in *adl1e* mutant guard cells on stomatal opening and closing responses, an in vitro system based on isolated epidermal peels was used in which the stomatal aperture could be measured. The epidermal peels were adapted for 3 h in the dark and then illuminated for 3 h. Stomata were of similar size in the epidermal peels of the WT and *adl1e* mutant, which exhibited maximal values of 4.3 and 4.2 μm, respectively (Fig. 4a). In the dark response after light-off, the stomatal apertures of both the mutant and WT cells decreased in a time-dependent manner. Interestingly, the stomata of the *adl1e* mutant closed faster than did those of the WT. Oligomycin strongly suppressed the dark response in both mutant and WT stomata. Fusicoccin (FC), a fungal elicitor, activates the plasma membrane H^+-ATPase (Palmgren 1998; Sze et al. 1999) and induces H^+-pumping by consumption of ATP, which provides the driving force for stomatal opening. Figure 4b showed that FC-induced stomatal opening in the dark increased to a greater extent in the *adl1e* mutant than in the WT over a 3-h period, which suggests a higher consumption of ATP in the mutant stomata. These results suggest that stomatal closure in the dark is an ATP-requiring response that is maintained by mitochondrial activity.

The *adl1e* mutant plants are reported to contain a T-DNA insert in the 14th exon (Jin et al. 2003). This mutation greatly

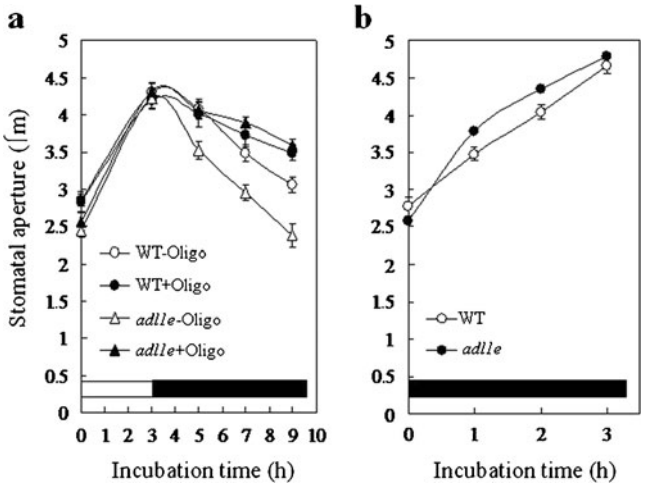

Fig. 4 Stomatal aperture following a light/dark transition and fusicoccin (FC)-induced stomatal opening in the dark in the epidermal peels of WT and *adl1e* mutant plants. **a** Epidermal peels were irradiated with white light (WL) (115 μmol m^{-2} s^{-1}) for 3 h (*white bar on x-axis*) and then incubated in the dark (*black bar on x-axis*). Oligomycin (Oligo, 5 μg ml^{-1}) was added to the suspension medium after the peels were placed in the dark. Data are the means ± SE (n=8) from five independent experiments (186≤(stomata)≤223). **b** FC was added to the suspension medium at a concentration of 10 μM. The epidermal peels were incubated in the dark for 3 h. Data are means ± SE (n=7) from three independent experiments (157≤(stomata)≤192)

increased cytosolic ATP content in MCPs and GCPs in the dark (Fig. 3). To elucidate the cytosolic ATP-dependent mechanism that induces stomatal closure during the light/dark transition, we assayed stomatal opening and closing responses in *adl1e* mutant leaves. Cytosolic ATP was required for the stomatal closing response to a dark stimulus (Fig. 4a). Since FC-induced H^+-pumping depends on cytosolic ATP, the rate of H^+-pumping may reflect the cytosolic concentration of ATP (Fig. 4b). Accordingly, we suggest that mitochondrial activity mediates stomatal closure in the dark.

In conclusion, our results suggest that cytosolic ATP maintains stomatal closure in darkness. If the ATP generated by mitochondria in the dark is used to power ion channels at the plasma membrane, the dark-induced stomatal closure is expected to be promoted by cellular mediator(s), such as Ca^{2+}/calmodulin-dependent protein phosphorylations (Cousson et al. 1995). It is also considered its association in the downstream of AtMYB61 (Liang et al. 2005), an R2R3-MYB transcription factor controlling stomatal aperture in the dark. However, the components of the dark signal pathway in guard cells remain to be elucidated.

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References

Assmann SM (1993) Signal transduction in guard cells. Annu Rev Cell Biol 9:345–375

Assmann SM, Simoncini L, Schroeder JI (1985) Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*. Nature 318:285–287

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Braidot E, Petrussa E, Vianello A, Macri F (1999) Hydrogen peroxide generation by higher plant mitochondria oxidizing complex I or complex II substrates. FEBS Lett 451:347–350

Choi SM, Jeong SW, Jeong WJ, Kwon SY, Park YI (2002) Chloroplast Cu/Zn-superoxide dismutase is a highly sensitive site in cucumber leaves chilled in the light. Planta 216:315–324

Cousson A, Cotelle V, Vavasseur A (1995) Induction of stomatal closure by vanadate or a light/dark transition involves Ca^{2+}-calmodulin-dependent protein phosphorylations. Plant Physiol 109:491–497

Dudkina NV, Jesco Heinemeyer J, Sunderhaus S, Boekema EJ, Braun H-P (2006) Respiratory chain supercomplexes in the plant mitochondrial membrane. Trend Plant Sci 11:232–239

Genty B, Briantais JM, Barker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92

Goh C-H, Schreiber U, Hedrich R (1999) New approach of monitoring changes in chlorophyll *a* fluorescence of single guard cells and protoplasts in response to physiological stimuli. Plant Cell Environ 22:1057–1070

Goh C-H, Dietrich P, Steinmeyer R, Schreiber U, Nam HG, Hedrich R (2002) Parallel recordings of photosynthetic electron transport and K+-channel activity in single guard cells. Plant J 32:623–630

Goh C-H, Jung KH, Roberts SK, McAinsh MR, Hetherington AM, Park YI, Suh K, An G, Nam HG (2004) Mitochondria provide the main source of cytosolic ATP for activation of outward-rectifying K^+ channels in mesophyll protoplast of chlorophyll-deficient mutant rice seedlings. J Biol Chem 279:6874–6882

Goh C-H, Lee DJ, Bae HJ (2009) Gibberellic acid of *Arabidopsis* regulates the abscisic acid-induced inhibition of stomatal opening in response to light. Plant Sci 176:136–142

Goh C-H, Satoh K, Kikuchi S, Kim S-C, Ko S-M, Kang HG, Jeon J-S, Kim CS, Park YI (2010) Mitochondrial activity in illuminated leaves of chlorophyll-deficient mutant rice (*OsCHLH*) seedling. Plant Biotech Rep 4:281–291

Hong Z, Bednarek SY, Blumwald E, Hwang I, Jurgens G, Menzel D, Osteryoung KW, Raikhel NV, Shinozaki K, Tsutsumi N et al (2003a) An unified nomenclature for Arabidopsis dynamin-related large GTPases based on homology and possible functions. Plant Mol Biol 53:261–265

Hong Z, Geisler-Lee CJ, Zhang Z, Verma DP (2003b) Phragmoplastin dynamics: multiple forms, microtubule association and their roles in cell plate formation in plants. Plant Mol Biol 53:297–312

Iino M, Ogawa T, Zeiger E (1985) Kinetic properties of the blue-light response of stomata. Proc Natl Acad Sci USA 82:8019–8802

Jin JB, Bae HJ, Kim SJ, Jin YH, Goh CH, Kim DH, Lee YJ, Tse YC, Jiang L, Hwang IW (2003) The Arabidopsis dynamin-like proteins ADL1C and ADL1E play a critical role in mitochondrial morphogenesis. Plant Cell 15:2357–2369

Kang BH, Busse JS, Bednarek SY (2003) Members of the Arabidopsis dynamin-like gene family, ADL1, are essential for plant cytokinesis and polarized cell growth. Plant Cell 15:899–913

Karlsson PE, Schwartz A (1988) Characterization of the effects of metabolic inhibitors, ATPase inhibitors, and a potassium channel blocker on stomatal opening and closing in isolated epidermis of *Commelina communis* L. Plant Cell Environ 11:165–172

Konopka CA, Bednarek SY (2008) Comparison of the dynamics and functional redundancy of the Arabidopsis dynamin-related isoforms DRP1A and DRP1C during plant development. Plant Physiol 147:1590–1602

Liang YK, Dubos C, Dodd IC, Holroyd GH, Hetheringtin AM, Campbell MM (2005) AtMYB61, an R2R3-MYB transcription factor controlling stomatal aperture in *Arabidopsis thaliana*. Curr Biol 15:1201–1206

Manfredi G, Yang L, Gajewski CD, Mattiazzi M (2002) Measurements of ATP in mammalian cells. Methods 26:317–332

Ogawa T, Ishikawa H, Shimada K, Shibata K (1978) Synergistic action of red and blue light and action spectrum for malate formation in guard cells of *Vicia faba* L. Planta 142:61–65

Palmgren MG (1998) Proton gradients and plant growth: role of the plasma membrane H^+-ATPase. Adv Bot Res 28:1–70

Pandey S, Wang X-W, Coursol SA, Assmann SM (2001) Preparation and application of *Arabidopsis thaliana* guard cell protoplasts. New Phytol 153:517–526

Pastore D, Di Martino C, Bosco G, Passarella S (1996) Stimulation of ATP synthesis via oxidative phosphorylation in wheat mitochondria irradiated with helium-neon laser. Biochem Mol Biol Int 39:149–157

Philip H, John B (2002) Ultraviolet radiation has no effect on respiratory oxygen consumption or enhanced post-illumination respiration in three species of microalgae. J Photochem Photobiol 68:109–116

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta 975:384–394

Praefcke GJK, Praefcke, McMahon HT (2004) The dynamin superfamily: universal membrane tubulation and fission molecules? Nat Rev Mol Cell Biol 5:133–147

Schmidt C, Schelle I, Liao YJ, Schroeder JI (1995) Strong regulation of slow anion channels and abscisic acid signaling in guard cells by phosphorylation and dephosphorylation events. Proc Natl Acad Sci USA 92:9535–9539

Schwarz M, Schroeder JI (1998) Abscisic acid maintains S-type anion channel activity in ATP-depleted *Vicia faba* guard cells. FEBS Lett 428:177–182

Shaw JM, Nunnari J (2002) Mitochondrial dynamics and division in budding yeast. Trends Cell Biol 12:178–184

Shimazaki K, Gotow I, Sakati I, Kondo N (1983) High respiratory activity of guard cell protoplasts from *Vicia faba*. Plant Cell Physiol 24:1049–1056

Shimazaki K, Lino M, Zeiger E (1986) Blue light-dependent proton extrusion of guard cell protoplasts of *Vicia faba*. Nature 319:324–326

Stone S, Ganf G (1987) The influence of previous light history on the respiration of four species of freshwater phytoplankton. Arch Hydrobiol 91:435–462

Sze H, Li X, Palmgren MG (1999) Energization of plant cell membranes by H^+–pumping ATPases: regulation and biosynthesis. Plant Cell 11:677–689

Tominaga M, Kinoshita T, Shimazaki K (2001) Guard cell chloroplasts provide ATP required for H^+ pumping in the plasma membrane and stomatal opening. Plant and Cell Physiol 42:795–802

Vani T, Raghavendra AS (1994) High mitochondrial activity but incomplete engagement of the cyanide-resistant alternative pathway in guard cell protoplasts of pea. Plant Physiol 105:1263–1268

Verma DPS, Hong Z (2005) The ins and outs in membrane dynamics: tubulation and vesiculation. Trends Plant Sci 10:159–165

Walker D (1987) The use of the oxygen electrode and fluorescence probes in simple measurements of photosynthesis. Oxygraphics, Sheffield

Ward JM, Pei ZM, Schroeder JI (1995) Roles of ion channels in initiation of signal transduction in higher plants. Plant Cell 17:833–844

Wong ED, Wagner JA, Gorsich SW, McCaffery JM, Shaw JM, Nunnari J (2000) The dynamin-related GTPase, Mgm1p, is an intermembrane space protein required for maintenance of fusion competent mitochondria. J Cell Biol 151:341–352

Xie Z, Chen Z (1999) Salicylic acid induces rapid inhibition of mitochondrial electron transport and oxidative phosphorylation in tobacco cells. Plant Physiol 120:217–225