

Light Regulation of Stomatal Movement

Ken-ichiro Shimazaki,¹ Michio Doi,²
Sarah M. Assmann,³ and Toshinori Kinoshita^{1,4}

¹Department of Biology, Faculty of Science, ²Research and Development Center for Higher Education, Kyushu University, Ropponmatsu, Fukuoka, 810-8560, Japan; email: kenrcb@mbox.nc.kyushu-u.ac.jp, doircb@mbox.nc.kyushu-u.ac.jp

³Department of Biology, Pennsylvania State University, University Park, Pennsylvania 16802-5301; email: sma3@psu.edu

⁴PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama, Japan; email: toshircb@mbox.nc.kyushu-u.ac.jp

Annu. Rev. Plant Biol. 2007. 58:219–47

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi:
10.1146/annurev.arplant.57.032905.105434

Copyright © 2007 by Annual Reviews.
All rights reserved

First published online as a Review in Advance on
January 5, 2007

1543-5008/07/0602-0219\$20.00

Key Words

blue light, chloroplasts, guard cells, phototropin, plasma membrane H⁺-ATPase, stomata

Abstract

Stomatal pores, each surrounded by a pair of guard cells, regulate CO₂ uptake and water loss from leaves. Stomatal opening is driven by the accumulation of K⁺ salts and sugars in guard cells, which is mediated by electrogenic proton pumps in the plasma membrane and/or metabolic activity. Opening responses are achieved by coordination of light signaling, light-energy conversion, membrane ion transport, and metabolic activity in guard cells. In this review, we focus on recent progress in blue- and red-light-dependent stomatal opening. Because the blue-light response of stomata appears to be strongly affected by red light, we discuss underlying mechanisms in the interaction between blue-light signaling and guard cell chloroplasts.

Contents

INTRODUCTION	220
BLUE-LIGHT RESPONSE	
OF STOMATA	221
Properties	221
Electrogenic H ⁺ Pump Drives K ⁺	
Uptake and Stomatal	
Opening	222
Entity of H ⁺ Pump and	
Regulation	224
Blue-Light Receptors	225
Biochemical Evidence for	
Phototropins as Blue-Light	
Receptors	226
Blue-Light Signaling in Guard	
Cells	227
Energy Source for	
Blue-Light-Dependent Proton	
Pumping	229
Metabolism Supporting	
Blue-Light-Dependent	
Stomatal Opening	230
Role of Blue-Light-Specific	
Stomatal Opening	231
Interaction of Blue-Light Signaling	
with Abscisic Acid	231
Other Photoreceptors	232
RED-LIGHT RESPONSE	
OF STOMATA	233
SYNERGISTIC EFFECT OF BLUE	
AND RED LIGHT IN	
STOMATAL OPENING	233
CONCLUDING REMARKS	236

INTRODUCTION

Stomata regulate gas exchange between plants and atmosphere, optimize photosynthetic CO₂ fixation, and minimize transpirational water loss (8, 17, 144, 189, 193). Stomata move rapidly to adjust plants to the ever-changing environment. The opening of stomata is driven by the accumulation of K⁺ salts (37, 42, 64, 191) and/or sugars (124, 174) in guard cells, which results in a decrease

in water potential and subsequent water uptake. Turgor elevation from water uptake increases guard cell volume, which widens stomatal apertures because of the asymmetric positioning of microfibrils in the cell wall. The volume increase requires an increase in surface area of the guard cell plasma membrane, and this needed area is provided by the internal membranes of guard cells (162). Guard cells possess a number of small vacuoles in the closed state of stomata (117). Such small vacuoles fuse with each other and generate bigger vacuoles during stomatal opening (43). Prior to these processes, a large number of ions move from the cytosol to the vacuole via channels and pumps in the tonoplast (186). Stomatal closure is caused by the release and/or removal of osmotica from guard cells under drought, darkness, elevated CO₂, or low humidity, resulting in the internalization of the plasma membrane and the generation of small vacuoles.

Stomatal opening is induced by light, including blue and red light, and distinct mechanisms underly stomatal opening in response to these different wavelengths (193). Blue light acts as a signal and red light as both a signal and an energy source. Blue light activates the plasma membrane H⁺-ATPase (21, 76), hyperpolarizing the membrane potential with simultaneous apoplast acidification, and drives K⁺ uptake through voltage-gated K⁺ channels. Red light drives photosynthesis in mesophyll and guard cell chloroplasts and decreases the intercellular CO₂ concentration (*C_i*). Red-light-induced stomatal opening may result from a combination of guard cell response to the reduction in *C_i* and a direct response of the guard cell chloroplasts to red light (132, 151, 183). In the afternoon, sugars also accumulate in guard cells as osmotica and maintain stomatal opening (174). The accumulation of positively charged K⁺ ions in guard cells must be compensated by anions, mainly in the form of the organic acid malate²⁻ (189). Malate forms in response to weak blue light under a red-light background, and the formation does not occur without

red light (109, 110). Guard cell chloroplasts are responsible for malate formation (151), and the chloroplasts also act as a reservoir for starch and catabolize it as a precursor of malate (183, 189). Guard cells also utilize Cl^- and NO_3^- as counterions for K^+ , although such utilization varies with plant species and growth conditions (51).

In this review, we focus on recent progress concerning light-stimulated stomatal opening. On the basis of the highly specialized metabolism in guard cells, which facilitates the rapid transport of ions across membranes, a synergistic effect between blue and red light on stomatal opening has been proposed. For other stomatal responses, particularly stomatal closure responses induced by the phytohormone abscisic acid (ABA) under drought, please refer to other recent reviews on this subject (11, 17, 35, 56, 132, 144).

BLUE-LIGHT RESPONSE OF STOMATA

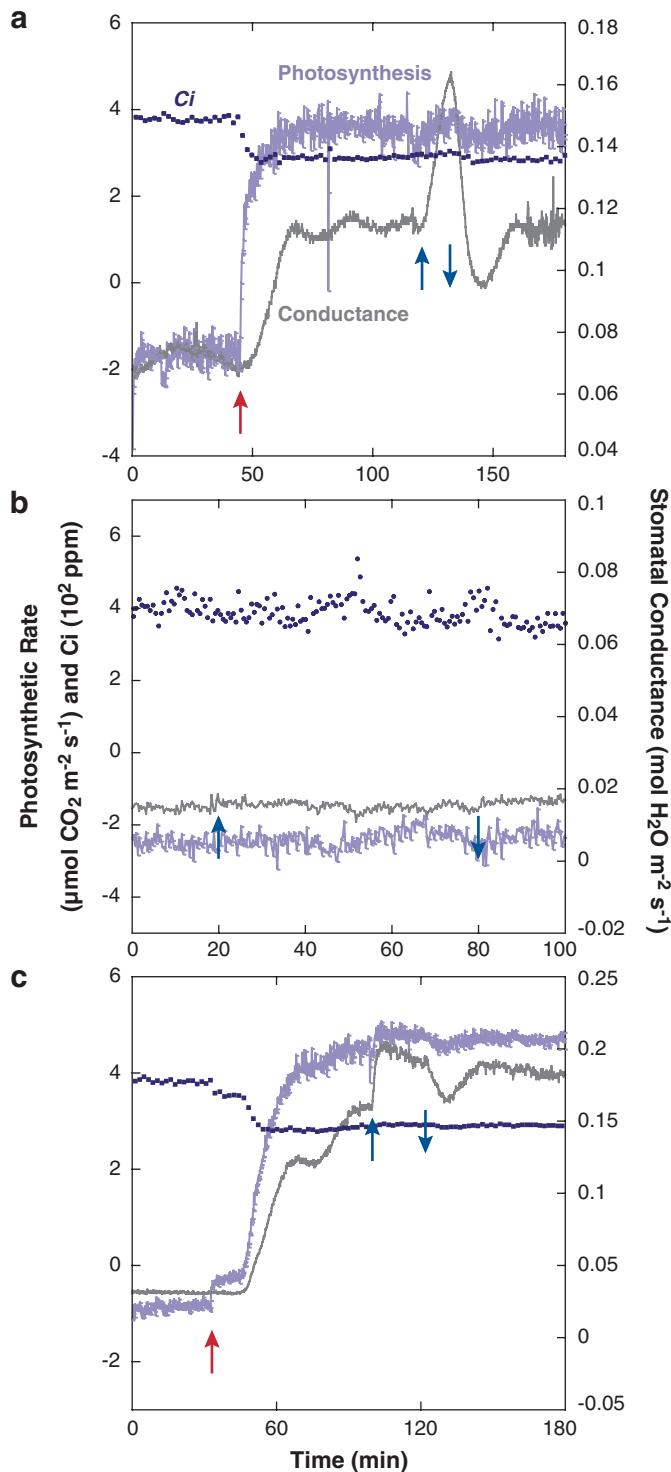
Properties

Blue-light-specific stomatal opening is found in a number of C_3 and C_4 plants under strong background red light, and in facultative Crassulacean acid metabolism plants functioning in the C_3 , but not the CAM, mode (89).

In intact leaves, blue light, on a quantum basis, is several to 20 times more effective than red light in opening stomata (19, 59, 71, 151, 152). The high sensitivity of this blue-light response becomes prominent in the presence of background red light. Simultaneous measurements of stomatal conductance, photosynthetic CO_2 fixation, and intercellular CO_2 concentration (C_i) in leaves of *Arabidopsis thaliana* and rice (*Oryza sativa* L. cv, Taichung 65) plants in response to light are presented in **Figure 1**. When a leaf kept in the dark for 1 h was illuminated with strong red light ($600 \mu\text{mol m}^{-2} \text{s}^{-1}$), photosynthetic CO_2 fixation occurred instantly with a sharp drop in C_i to 250–270 ppm, followed by a gradual in-

crease in photosynthetic rate for 20 min, until a steady state was achieved. Stomata showed a gradual increase in stomatal conductance with a short lag time, and conductance reached a maximum within 20 min, then showed small fluctuations (**Figure 1a**). Weak blue light ($5 \mu\text{mol m}^{-2} \text{s}^{-1}$), superimposed on the red light for 10 min, elicited rapid stomatal opening, with a threefold faster rate than that caused by red light (151). The opening responses were observed repeatedly in response to blue light, and the magnitude of the responses showed a slight decrease from morning to afternoon (28). Stomata showed virtually no opening in response to the weak blue-light stimulus in the absence of red light (**Figure 1b**). These properties are in accord with those shown in previous reports on other plant species (63, 86, 151). When the rice leaf was illuminated with red light, photosynthetic CO_2 fixation increased instantly but remained at a low level for 10 min due to the low stomatal conductance, and then exhibited a large gradual increase (**Figure 1c**). The gradual increase in photosynthesis resulted from the removal of stomatal limitation, because the increase in CO_2 fixation paralleled that in stomatal conductance of this phase. Weak blue light superimposed on the red light induced a very rapid increase in the stomatal aperture in this plant species (**Figure 1c**), as has also been shown for other monocots, such as wheat (70, 71) and sugarcane (9).

Blue light acts as a signal, and even a short period of light (pulse, 30–60 s) induces stomatal opening, which is sustained for more than 10 min after the pulse (63). Blue-light-specific stomatal responses can be induced by illuminating the leaves with blue light superimposed on high-intensity red light, in which photosynthesis was not further activated by the blue-light stimulus (110, 148). The magnitude of the response was proportional to the photon flux of blue light and reached saturation. Once a single saturating pulse was given to the leaf, the responsiveness to a second pulse was greatly reduced when the second pulse was immediately



applied, and the responsiveness was gradually restored with a half-life time ($T_{1/2}$) of 9 min in *Commelina communis* (63). The blue-light receptor was suggested to exist in two interconvertible forms: one physiologically active and the other inactive (63). The conversion from the inactive form to the active form was a light-induced fast reaction and the active form gradually returned to the inactive form via a thermal reaction. These properties seem to be attributable to the molecular properties of the responsible blue-light receptors, which were discovered later (60, 72, 138).

Electrogenic H^+ Pump Drives K^+ Uptake and Stomatal Opening

Medium acidification by guard cells in *Vicia* epidermal peels was reported, and the

Figure 1

Stomatal conductance, photosynthetic CO_2 fixation, and intercellular CO_2 concentration in response to light in leaves of *Arabidopsis thaliana* (a, b) and *Oryza sativa* (c). Red upward arrows indicate continuous irradiation of the leaf with a strong red light ($600 \mu\text{mol s}^{-1} \text{ m}^{-2}$). Upward and downward blue arrows indicate onset and termination of irradiation, respectively, with a weak blue light ($5 \mu\text{mol s}^{-1} \text{ m}^{-2}$). (a) When an *Arabidopsis* leaf was illuminated with red light, stomatal conductance gradually increased and reached a steady state within 20 min. Photosynthetic CO_2 fixation showed an initial rapid increase with a subsequent gradual increase. This gradual increase is probably due to removal of stomatal limitation. When weak blue light was superimposed on red light, the stomatal conductance increased rapidly and decreased after turning off the blue light. Photosynthetic CO_2 fixation was only slightly enhanced by blue light. (b) When an *Arabidopsis* leaf was illuminated with weak blue light for 60 min in the absence of red light, neither stomatal conductance nor photosynthetic CO_2 fixation was increased. (c) When a rice leaf was illuminated with red light, stomatal conductance did not change for 10 min, whereas photosynthetic CO_2 fixation showed a rapid increase to a very low level. Upon the gradual increase in stomatal conductance, photosynthetic CO_2 fixation showed a parallel increase. When the leaf was irradiated with weak blue light superimposed on red light, a very rapid increase in stomatal conductance was observed.

acidification was required for K^+ uptake and stomatal opening (126). The H^+ release from guard cells was stimulated by light and was inhibited by vanadate (46). Zeiger & Hepler (197) found that isolated guard cell protoplasts of onion (*Allium cepa*) increased their volume in response to blue light, which is indicative of stomatal opening. This finding demonstrated that all components responsible for blue-light-specific stomatal opening are localized in guard cells. Using a large number of guard cell protoplasts from *Vicia faba*, it was found that guard cells extruded H^+ in response to a pulse of blue light (157). The properties of H^+ extrusion were very consistent with those of blue-light-induced stomatal opening in intact leaves (63). The H^+ extrusion was mediated by electrogenic pumping, and patch clamp experiments demonstrated that blue light induced a transient membrane hyperpolarization (12). These results indicate that blue light increases the inside-negative electrical potential across the plasma membrane by activating the electrogenic proton pump (Figure 2). Because acidification of the external medium by epidermal strips and swelling of guard cell protoplasts by blue light were inhibited by vanadate, an inhibitor of the plasma membrane H^+ -ATPase (5, 46), and the blue-light-stimulated electrogenic current required cytosolic ATP (12), the H^+ pump was proposed to be the plasma membrane H^+ -ATPase. In this initial report, the blue-light-induced H^+ current across the plasma membrane as measured by whole-cell patch clamp was not of the magnitude required for sufficient K^+ accumulation in guard cells to drive stomatal opening. This may be due to loss of essential cytoplasmic components in the whole-cell configuration, and a much larger current was obtained in a slow whole-cell configuration (8, 143). A sufficiently large current was also measured in whole-cell patch clamp experiments (177) and in intact *Vicia* guard cells (135). A blue-light-sensitive electron transport chain in the guard cell plasma membranes, which releases H^+ to the medium, was hypothesized as an alternative

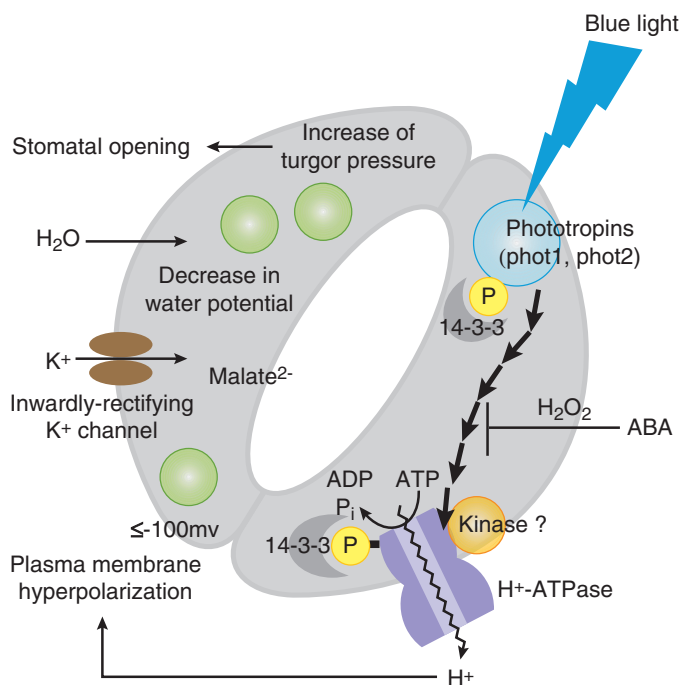


Figure 2

Processes of stomatal opening in response to blue light.

candidate mechanism (45, 125). Redox regulation of the blue-light-activated H^+ pump may be possible (181, 183), although manipulation of cytosolic NADH and NADPH concentrations did not alter the magnitude of blue-light-stimulated pump current (177).

After discovery of the blue-light-induced H^+ pump, voltage-gated K^+ channels, activated by membrane hyperpolarization, were discovered in the guard cell plasma membrane of the same plant species (146). The channels (inward-rectifying K^+ channels, K^+_{in}) function to take up K^+ when guard cells are hyperpolarized to values more negative than the equilibrium potential for K^+ (Figure 2). K^+_{in} channels require ATP for the activation and maintenance of their activity (47, 190), and are sensitive to inhibition by Ca^{2+} (34, 145). The first genes for K^+_{in} channels to be cloned from *Arabidopsis* were designated as *KAT1* (6) and *AKT1* (149). These genes encoded K^+_{in} channels, as determined by expressing the genes in *Xenopus oocytes* and evaluating the

resultant currents (104, 141, 184). Subsequent research showed that in addition to *KAT1* and *AKT1*, the *Arabidopsis* K⁺ channel genes *AKT2/3*, *AtKC1*, and *KAT2* are also expressed in guard cells (107, 170). *KST1* and *KPT1* are highly expressed in guard cells of potato and poplar plants, respectively (84, 104, 123). Introducing a dominant-negative nonfunctional *KAT1* gene lowered the inward K⁺ current by 75% in guard cells and reduced light-induced stomatal opening by 40% (82), suggesting that the inward current largely requires *KAT1*, although other K⁺_{in} channels are also functional (170).

Entity of H⁺ Pump and Regulation

The pump was subsequently demonstrated to be a plasma membrane H⁺-ATPase that is activated by phosphorylation (76). H⁺-ATPase activity in guard cell protoplasts was increased by blue light, and the activity was closely correlated with the rate of H⁺ pumping. Levels of phosphorylation of the H⁺-ATPase were increased by blue light and the amount of increase was proportional to the rate of H⁺ pumping. The phosphorylation occurred exclusively on the C terminus of the H⁺-ATPase, with subsequent binding of a 14-3-3 protein to the phosphorylated C terminus, which has been shown to function as an autoinhibitory domain of the H⁺-ATPase (119). Phosphorylation of the Ser and Thr residues within this domain promotes H⁺-ATPase activity (165), indicating that a serine/threonine protein kinase is involved in this regulation (Figure 2).

Binding of a 14-3-3 protein to the H⁺-ATPase was absolutely required for its activation; phosphorylation alone was not sufficient to activate the H⁺-ATPase (78). Four isoforms of 14-3-3 proteins (Vf14-3-3a, b, c, and d) were detected in *Vicia* guard cells, and mass analysis of the 14-3-3 protein that had bound to the H⁺-ATPase in vivo identified Vf14-3-3a as a specific isoform binding to the plasma membrane H⁺-ATPase (33). The isoform Vf14-3-3a had a higher binding affini-

ty to the H⁺-ATPase than Vf14-3-3b, and Vf14-3-3a might more effectively activate the H⁺-ATPase than Vf14-3-3b. The binding site of a 14-3-3 protein was determined to be the penultimate Thr residue in the C terminus by competition experiments with synthetic phosphopeptides (78). The site identified by this method was the same as that determined by genetic analysis of fusicoccin-dependent activation of the plasma membrane H⁺-ATPase in spinach leaves (169).

Because the plasma membrane H⁺-ATPase shows primary and pivotal functions in many plant tissues by coupling with various carriers and channels, the regulatory mechanisms of these enzymes have been extensively investigated using the fungal toxin fusicoccin (119, 168). However, activation of the H⁺-ATPase by this toxin occurs irreversibly and such mechanisms might not be the same as those occurring in vivo. Guard cells are the ideal systems to manipulate the activity of this enzyme in vivo, and the associated regulatory mechanisms can be investigated under physiological conditions (76).

There are several to 10 isoforms of the plasma membrane H⁺-ATPase in plant cells, and 11 functional H⁺-ATPases are present in *Arabidopsis* (119). Tissue-specific expression of the H⁺-ATPase isoforms has been observed in several organs, including *AHA3* in phloem companion cells (26), *AHA9* in anther cells (58), and *AHA4* in roots (185). Therefore, it is possible that the H⁺-ATPase(s), which is specifically activated by blue light, is expressed in guard cells in a cell-specific manner. In *Vicia* guard cells, isoproteins of VHA1 (*Vicia* H⁺-ATPase1) and VHA2 were activated by blue light (76). However, these isoproteins were also expressed in roots, leaves, stems, and flowers (55, 106). These observations suggest that guard cells have an additional blue-light signaling system that is cell specific, and the light signal is delivered to the usual H⁺-ATPase(s) via this system. Interestingly, all 11 isogenes of AHA were expressed in guard cells, whereas only 4 isogenes were expressed in mesophyll cells of *Arabidopsis* plants

(180). In pulvinar motor cells of *Phaseolus vulgaris*, the plasma membrane H^+ -ATPase exists in a highly active and heavily phosphorylated state in the dark, and is inactivated by dephosphorylation via phototropin-mediated reactions (66), suggesting that the blue-light signaling systems are flexible and can be modulated by cell-specific regulatory mechanisms. The H^+ -ATPase inactivation caused water loss from the motor cells and resulted in leaf movement.

Blue-Light Receptors

Flavin and zeaxanthin, a component of the xanthophyll cycle (108), are candidate chromophores for the blue-light receptor of guard cells on the basis of action spectra for stomatal opening in intact leaves and malate synthesis in epidermal peels (71, 110, 199). The sensitivity of stomata to blue light increased with their zeaxanthin content (198), and inhibition of zeaxanthin formation suppressed blue-light-stimulated stomatal opening. The zeaxanthin-less mutant of *Arabidopsis*, *npq1*, failed to respond to blue light (40). Strong green light suppressed the blue-light-dependent stomatal opening in epidermal strips of *Arabidopsis* (39), and this effect was also found in isolated epidermal peels from light-treated leaves (173). These results suggest the involvement of zeaxanthin in the blue-light response of stomata. However, further confirmation is needed because the stomatal aperture responses reported for the *npq1* mutant could not be reproduced in leaves, epidermes, or guard cell protoplasts (29, 72, 180).

In 1997, a blue-light receptor for phototropism was identified by the Briggs group (60). The photoreceptor has two Light, Oxygen, Voltage (LOV) domains in the N terminus and a serine/threonine protein kinase in the C terminus, and undergoes autophosphorylation in response to blue light (21, 24). The LOV domains (LOV1 and LOV2) function as binding sites for flavin mononucleotide and absorb blue light (25). A homolog of this protein was found (67) and

these proteins were initially called nonphototropic hypocotyl (NPH1) and nonphototropic hypocotyl like (NPL1), and were subsequently renamed phototropins 1 (*phot1*) and 2 (*phot2*), respectively (20). Later, *phot2* was demonstrated to be the photoreceptor that mediates the chloroplast avoidance response to high-intensity light (68, 69).

The absorption spectrum of the LOV2 domain has peaks at 378 nm in the UV-A region, and 447 nm and 475 nm in the blue region (138). The action spectrum for stomatal opening in wheat leaves closely matches the absorption spectrum of the LOV domains (71). It was thus plausible to hypothesize that phototropins function as the blue-light receptors in guard cells (**Figure 2**). However, in the *Arabidopsis phot1* mutant, transpiration in the leaf still responded to blue light (87). Thus, *phot2* might act as a blue-light receptor in guard cells, or *phot1* and *phot2* might function redundantly in the cells. To resolve this issue, the stomatal response was investigated using a single mutant of *phot2*, and the *phot1 phot2* double mutant of *Arabidopsis*. In the epidermal peels of *Arabidopsis*, stomata in the *phot1 phot2* double mutant did not open in response to blue light, but stomata opened in the *phot1*, *phot2*, and *npq1* single mutants (72). Epidermal strips from the double mutant were unable to extrude H^+ in response to blue light. In this mutant, no change was found in the amount of plasma membrane H^+ -ATPase, and the mutant could respond to fusicoccin by opening stomata. Thus, the lack of stomatal opening and H^+ extrusion by blue light is due to an impairment in blue light perception. Stomata in the *phot2* mutant retain more sensitivity to blue light than those of *phot1* in the opening response.

Involvement of phototropins in stomatal response was confirmed by the complementation of the *phot1 phot2* double mutant with the *PHOT1* gene (28): Stomatal conductance in leaves of the *phot1 phot2* double mutant did not increase following blue-light irradiation, and the response was restored in the transformant. *Chlamydomonas reinhardtii* phototropin

(Crphot), which acts as a blue-light receptor in the sexual life cycle of algae (61), also restored stomatal response when the *phot1 phot2* double mutant was transformed with *CrPHOT* (113).

Biochemical Evidence for Phototropins as Blue-Light Receptors

There are at least two *Vicia faba* phototropins, 1a and 1b (Vfphot1a and 1b), with molecular masses of 125 kDa, and both are expressed in guard cells. If phototropins function upstream of the H⁺-ATPase, then they should be phosphorylated more quickly than the H⁺-ATPase. Simultaneous determination of the phosphorylation levels of phototropins and the H⁺-ATPase in *Vicia* guard cells revealed that this was indeed the case (73). The maximum phosphorylation levels of Vfphot1 and the H⁺-ATPase appeared around 1 and 5 min, respectively, after the onset of blue light. When phototropin phosphorylation was inhibited by diphenyleneiodonium chloride (DPI), a flavoprotein inhibitor, or by protein kinase inhibitors (K-252a and staurosporine), the H⁺-ATPase phosphorylation was inhibited to the same degree as that of phototropins. Phosphorylation of phototropins and the H⁺-ATPase showed similar photon flux dependency. Phototropin 1 is associated with the plasma membrane in guard cells (137), where the H⁺-ATPase is also localized, and this is consistent with the idea that phot1 plays a role in blue-light-induced stomatal opening. Thus, biochemical and pharmacological evidence indicate that phototropins act upstream of the plasma membrane H⁺-ATPase (**Figure 2**).

The binding of 14-3-3 proteins to target proteins can modify enzyme activity, change intracellular localization, and generate a scaffold for the interaction with other proteins (15, 36, 62, 78, 101). Phototropin binds reversibly to a 14-3-3 protein upon its autophosphorylation in guard cells (73). The 14-3-3-phototropin complex might confer a signaling state to phototropins, which then could

transmit the light signal to downstream elements (**Figure 2**). Binding of a 14-3-3 protein to phototropin was also seen in the tissues of both etiolated seedlings and green leaves.

Binding of a 14-3-3 protein to target proteins was phosphorylation-dependent in general. However, the amount of 14-3-3 protein bound did not parallel the levels of phototropin phosphorylation. The maximum level of 14-3-3 binding in guard cells was reached faster than that of phosphorylation, and dissociation of a 14-3-3 protein from phototropin was also faster than the dephosphorylation of phototropin. This is probably because phototropins are phosphorylated on multiple sites (139, 163), and only some of these sites act as the binding sites for 14-3-3 protein; these sites appeared to be phosphorylated and dephosphorylated faster than other sites. Because the binding sites of 14-3-3 protein had phospho-Ser consensus motifs, including R/KXXpSXP, R/XXXpSXP, and R/XXpS/T (1, 105), the binding sites were determined by substituting all candidate Ser residues in phototropins. The binding sites were located in the hinge region between LOV1 and LOV2 in *Vicia* phototropins (73). Two phosphorylation sites were determined to be localized in the N terminus and six in the hinge region between LOV1 and LOV2 in *Avena sativa* phot1 by combining *in vitro* and *in vivo* phosphorylation using protein kinase A (139). The phosphorylation sites had different blue-light sensitivities.

Phototropin in etiolated seedlings underwent phosphorylation by blue light and was dephosphorylated gradually under darkness (118), and the long-lived phosphorylated state could act as the desensitized state of the photoreceptor (163). The recovery of sensitivity to blue light in phototropism occurred with a kinetics similar to that for recovery from the phosphorylated state. Essentially the same desensitization process was found in the blue-light response of stomata. The responsiveness of stomata to a second blue-light pulse decreased when guard cells were irradiated immediately after a first pulse, and sensitivity was

gradually restored during the dark interval (63, 73, 157).

Blue-Light Signaling in Guard Cells

The signaling mechanism from phototropins to the plasma membrane H⁺-ATPase is largely unknown. However, several signaling molecules appear to be present between phototropins and the H⁺-ATPase (**Figure 2**).

14-3-3 proteins. The 14-3-3 proteins are involved in blue-light signaling in stomata as regulators of both phototropins and H⁺-ATPase. A 14-3-3 protein functions as an activator of the plasma membrane H⁺-ATPase via phosphorylation of the H⁺-ATPase in response to blue light (33, 78). A 14-3-3 protein also binds phototropin upon its phosphorylation, and the binding appears to precede the activation of the plasma membrane H⁺-ATPase in guard cells (73). However, the functional significance of the phototropin-14-3-3 protein complex has not been determined.

RPT2. Root Phototropism 2 (RPT2), a member of a unique, plant-specific family of proteins (102), was suggested to act as a signal transducer from phot1 to the downstream components of the signaling chain in phototropism and stomatal opening, but was not involved in chloroplast movement (65). RPT2 interacted genetically and physically with phot1 but not phot2, and the *phot2 rpt2* double mutant lost the capacity for blue-light-induced stomatal opening in the epidermis (**Figure 3**). Because the determination of stomatal aperture in these experiments was performed only in the epidermis, other separate lines of evidence will be needed to confirm this result.

VfPIP. A protein interacting with *Vicia phot1a* was isolated from guard cells (32). The *Vicia faba* phot1a interacting protein (VfPIP) bound to the N terminus of Vfphot1a but not to Vfphot1b. The *VfPIP* transcript was predominantly expressed in guard cells.

VfPIP has sequence homology to dynein light chain and localizes to cortical microtubules. Vfphot1a seemed to exert its function through the microtubules, because treating guard cells with microtubule depolymerizing compounds resulted in partial inhibition of blue-light-stimulated stomatal opening and H⁺ extrusion. A recent investigation indicated that the microtubules in guard cells are organized in parallel, straight, and dense arrays by blue light and are not affected by red light (83). Reorganization of the microtubules was not stimulated by activating the H⁺-ATPase by fusicoccin. VfPIP may accelerate stomatal opening via phototropin-mediated organization and reorientation of microtubules (**Figure 3**).

Ca²⁺. Ca²⁺ was suggested to be responsible for blue-light signaling in guard cells by pharmacological tools. Calmodulin antagonists inhibited both blue-light-dependent H⁺ pumping and stomatal opening (158). The pumping was inhibited by verapamil, a Ca²⁺ channel blocker, albeit at high concentrations (160). The H⁺ pumping was inhibited reversibly by caffeine, which releases Ca²⁺ from intracellular stores, and by inhibitors of endoplasmic reticulum Ca²⁺-ATPase. Although stimulation of blue-light-induced acidification by external Ca²⁺ was reported in *Arabidopsis* epidermis (134), neither Ca²⁺ channel blockers nor change in external Ca²⁺ concentration affected H⁺ pumping in *Vicia* (154). Therefore, the Ca²⁺ that is thought to be required for stomatal opening might originate from intracellular Ca²⁺ stores, most likely from the endoplasmic reticulum. In accord with this possibility, the anion channels that facilitate uptake of Cl⁻ and malate²⁻ into the vacuole, as occurs during stomatal opening, were activated by CDPK, a Ca²⁺-activated protein kinase in guard cells (122).

Recent investigations demonstrated that phot1 activation elicited an increase in cytosolic Ca²⁺ in *Arabidopsis* and tobacco seedlings (16). The Ca²⁺ increase exhibited a transient change with a possible lag period of 3–6 s and

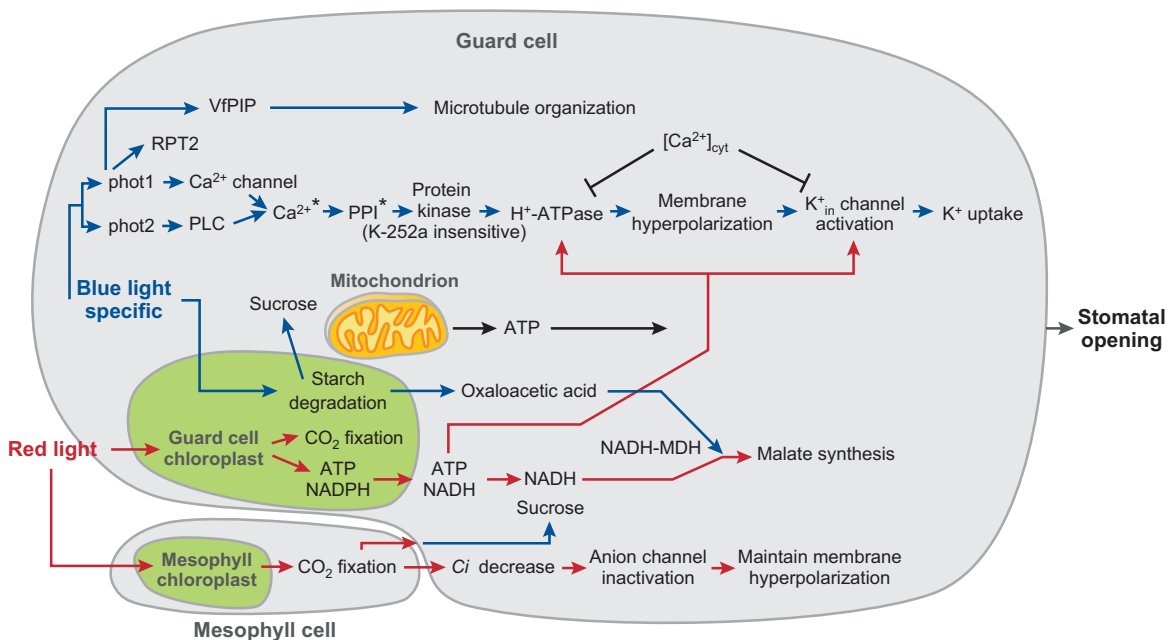


Figure 3

Cooperation of blue-light signaling and metabolism in guard cells for stomatal opening. Blue light is perceived by phot1 and phot2. The light signals are transmitted and activate the plasma membrane H⁺-ATPase, thereby inducing K⁺ uptake. Blue light also stimulates starch degradation. These responses are specific to blue light under normal conditions. Red light is absorbed by guard cells and mesophyll chloroplasts. Guard cell chloroplasts provide ATP and NADH in the cytosol, and mesophyll chloroplasts fix CO₂, thereby resulting in *C_i* decrease. The *C_i* decrease activates transport mechanisms that result in membrane hyperpolarization. These reactions are not specific to red light. Blue light is also absorbed by chloroplasts and has the same effect as red light, but the role of blue light through the chloroplasts is not depicted in this figure. * We placed Ca²⁺ upstream of protein phosphatase in this signaling pathway, but the order is speculative. K-252a, a broad inhibitor of Ser/Thr protein kinases; NADH-MDH, NADH-malate dehydrogenase; phot1, phototropin 1; phot2, phototropin 2; PLC, phospholipase C; RPT2, ROOT PHOTOTROPISM 2; VfPIP, *Vicia faba* phototropin interacting protein; PP1, type 1 protein phosphatase.

was sustained for 80 s after the pulse of blue light. The responsiveness to blue light was desensitized after the first pulse and gradually restored within 3–4 h. Based on mutant analysis, cry1 and cry2 do not participate in the signal transduction chain triggering this Ca²⁺ increase, whereas both phot1 and phot2 are involved and appear to mediate the cytosolic Ca²⁺ increase via calcium-permeable channels in the plasma membrane of mesophyll cells (16, 54, 167). phot2 may also function via phospholipase C-mediated Ca²⁺ release from intracellular stores (Figure 3), as inferred from assays with pharmacological inhibitors

of phospholipase C (PLC) (54). Phototropin-mediated Ca²⁺ increase was likely to be required for rapid inhibition of hypocotyl elongation (38), chloroplast movement (178), and phototropism (14, 16), and the rapid growth inhibition was phot1-dependent. However, note that an increase in cytosolic Ca²⁺ by blue light has not yet been reported in guard cells.

It has been well documented that Ca²⁺ is a second messenger for ABA-induced stomatal closure (17, 144). A high concentration of cytosolic Ca²⁺ caused stomatal closure or inhibited stomatal opening (42, 96, 147) through activation of anion channels, and inactivation

of the plasma membrane H^+ -ATPase and K^+ _{in} channels (74, 145). These results appear to conflict with the notion that Ca^{2+} is required for stomatal opening. ABA and a high concentration of external Ca^{2+} induced the oscillation of cytosolic concentration of Ca^{2+} with a definite period, and such oscillation was absolutely required for the maintenance of stomatal closure (3, 95). However, if cytosolic Ca^{2+} increased continuously without oscillation, or the intervals between oscillations were too short or too long (3), the Ca^{2+} response did not cause sustained stomatal closure (3, 4, 140). We note that, in other tissues, the Ca^{2+} increase elicited by blue light via phototropins did not show oscillations (16, 54, 167), and such a nonoscillatory increase might encode information opposite to that for stomatal closure.

Protein kinase. A serine/threonine protein kinase that directly phosphorylates the plasma membrane H^+ -ATPase is present in guard cells, and this kinase may be activated by blue light (76) (**Figure 2**). This kinase is unlikely to be phototropin itself, because the kinase was almost completely insensitive to kinase inhibitors that are effective against phototropin kinase activity (73, 77, 158, 167). A protein kinase activity that phosphorylates the H^+ -ATPase has been demonstrated in the plasma membrane of spinach leaves in vitro (169) (**Figure 3**).

Protein phosphatase. Okadaic acid and calyculin A inhibit both blue-light-dependent H^+ pumping and stomatal opening (75). This result implies that type 1 or type 2A protein phosphatase(s) mediate the signaling between phototropins and the plasma membrane H^+ -ATPase in guard cells. However, it has been unclear which type of protein phosphatase is involved in this signaling, because okadaic acid and calyculin A inhibit both phosphatases. Recent work indicates that type 1 protein phosphatase is responsible for this signaling (172) (**Figure 3**). When the dominant-negative form of type 1 protein

phosphatase or inhibitor-2, a proteinaceous-specific inhibitor of type 1 protein phosphatase, was expressed in *Vicia* guard cells via particle bombardment, stomatal opening by blue light was inhibited. Tautomycin, a preferential inhibitor of type 1 protein phosphatase in vivo, suppressed blue-light-induced phosphorylation of the H^+ -ATPase but did not affect the autophosphorylation of phototropins.

Energy Source for Blue-Light-Dependent Proton Pumping

Protons are pumped out at the expense of ATP by the plasma membrane H^+ -ATPase. It is likely that the energy for blue-light-dependent proton pumping is provided mainly by mitochondria and partly by chloroplasts (**Figure 3**), although the energy source may vary with the prevailing environment (13, 120, 189). Several chloroplasts and numerous mitochondria exist in guard cells, which exhibit high rates of respiration (2, 156, 182). Inhibited respiration reduced ATP levels drastically in guard cells, but the reduction was not severe in mesophyll cells (44, 156, 181). Stomatal opening specific to blue light was inhibited by potassium cyanide (KCN) and anoxia and partially affected by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of photosystem II (PSII) in photosynthetic electron transport (148). Oligomycin, KCN, and low oxygen tension largely reduced blue-light-dependent H^+ pumping, and DCMU partially inhibited these responses (93).

Guard cell chloroplasts synthesize ATP via cyclic and noncyclic photophosphorylation (161, 195) (**Figure 3**) and can be the main energy source when mitochondrial activity is reduced. In the presence of oligomycin, fusicoccin-induced H^+ pumping in guard cells was greatly reduced, whereas the addition of red light enhanced both H^+ pumping and stomatal opening, and this enhancement was eliminated by DCMU (179).

Metabolism Supporting Blue-Light-Dependent Stomatal Opening

Malate. The main events of blue-light-dependent stomatal opening are an activation of the plasma membrane H^+ -ATPase and K^+ uptake from the medium. To compensate the positively charged K^+ accumulated in guard cells, malate²⁻ is synthesized, and Cl^- and NO_3^- are taken up from the medium. Most plant species (except onion, which does not accumulate starch in guard cells) accumulate malate²⁻ preferentially over other anions. A close correlation has been obtained between stomatal opening and malate accumulation in guard cells (183, 189). Malate²⁻ can be produced through the degradation of starch in guard cells under blue light (Figure 3). The opening of stomata by blue light was severely impaired in *Arabidopsis* phosphoglucosyltransferase mutant plants, which did not accumulate starch in guard cell chloroplasts (88). However, this blue-light response was restored in the presence of high concentrations of Cl^- , possibly because Cl^- replaced malate²⁻ as a necessary counterion for K^+ . The mechanisms mediating uptake of anions, including Cl^- and NO_3^- , across the plasma membranes in guard cells are largely unknown, but it is likely that a cotransporter of H^+/Cl^- (NO_3^-) acts as the anion uptake carrier in guard cells.

Recent work indicated that *AtSTP1* (the H^+ -monosaccharide symporter gene) expression was quickly and largely upregulated in guard cells in darkness (166). The *AtSTP1* transporter may function in the guard cell import of apoplastic glucose derived from mesophyll cells under darkness and contribute to the accumulation of starch. The products of starch breakdown could be exported to the cytosol from guard cell chloroplasts and act as osmotica under the light (114, 116, 129), i.e., in the opposite direction as in mesophyll chloroplasts, where starch is broken down in darkness (164). Exported compounds might be converted to phosphoenolpyruvate (PEP)

in the cytosol, and PEP catalyzed by PEP carboxylase to produce OAA, which is then reduced to malate (189) (Figure 3). High activities of PEP carboxylase and NAD^+ malate dehydrogenase, required for these reactions, exist in guard cells.

Malate could be produced through light-activated $NADP^+$ malate dehydrogenase in guard cell chloroplasts (49), but the malate formed by this pathway marginally contributes to stomatal opening, because the activity of $NADP^+$ malate dehydrogenase is much lower than that of NAD^+ -malate dehydrogenase in guard cells (142, 189). Because guard cell chloroplasts are likely to overreduce electron acceptors due to their low Rubisco activity and resulting low CO_2 fixation, the light-activated $NADP^+$ -MDH may function as a malate valve to prevent damage to guard cells under strong sunlight (41).

Sucrose. Early investigations indicated that starch breakdown produced sugars that acted as principal osmotica for stomatal opening. This starch-sugar hypothesis had been replaced by the theory of guard cell osmoregulation via K^+ and its counterions (37, 42, 189). However, some studies have reported a parallel relationship between stomatal apertures and sucrose content in epidermal peels (128). Recent investigations demonstrate that sucrose accumulates in guard cells and replaces K^+ in the afternoon, thereby serving as a dominant osmoticum to maintain stomatal apertures (176). Sugar accumulates in guard cells under the light; however, the mechanism by which the sucrose accumulation occurs is largely unknown (183). Because guard cells are not connected with neighboring cells via plasmodesmata (188), two other pathways must operate in the sucrose accumulation: either uptake from the apoplast across the plasma membrane and/or production in these symplastically isolated cells. It is unlikely that photosynthetic CO_2 fixation in guard cell chloroplasts provides all of the sucrose, because the chlorophyll

content in guard cells is less than 2% of that of mesophyll cells on a cell basis and Rubisco activity is low on a chlorophyll basis (50, 115, 127, 153, 156). It is possible that sugars are produced in guard cells, but fast starch degradation is required for sufficient and continuous provision of sugars (112, 176) (**Figure 3**). Import of sucrose/hexose from the apoplast will likely occur (183). It is very likely that blue light stimulates sucrose/hexose uptake by coupling with a H^+ /sucrose or glucose symporter across the plasma membrane of guard cells through acidification of the apoplast by the H^+ -ATPase (**Figure 3**). In support of this idea, fusicoccin-induced activation of the H^+ -ATPase enhanced sucrose uptake in guard cell protoplasts (128, 130). Sucrose uptake by guard cells was reported in epidermal peels when they were overlaid onto mesophyll tissue (27). Because apoplastic sucrose reaches a high concentration in the daytime (91, 183), the H^+ /sucrose and H^+ /monosaccharide symporters could function coupled with the H^+ -ATPase. Transient upregulation of *AtSTP1* H^+ /monosaccharide symporter gene in the midday might contribute to guard cell-specific accumulation of sucrose (166). Expression of the sucrose transporter *AtSUC3* was demonstrated in guard cells of *Arabidopsis* (100).

Role of Blue-Light-Specific Stomatal Opening

The opening of stomata in response to blue light is fast and sensitive (151). Photosynthetic CO_2 fixation starts upon illumination and reaches maximum levels within several minutes. In general, stomatal opening occurs more slowly than photosynthesis, requiring more than 20 min to reach the maximum aperture in many plant species. At dawn, sunlight is rich in blue light, and the consequent rapid stomatal opening would enhance CO_2 uptake for photosynthesis (194).

Blue-light-induced stomatal opening is mediated via phototropins and enhances photosynthetic CO_2 fixation by removing

the stomatal limitation to CO_2 entry. Phototropins also mediate phototropic bending, chloroplast accumulation, leaf expansion, and leaf movement (21), and these responses maximize photosynthetic electron transport by improving the efficiency of light capture. Thus, the blue-light response of stomata optimizes photosynthesis in increasing the CO_2 provision for stromal reactions in coordination with these phototropin-mediated thylakoid reactions. Such functions of phototropins were demonstrated by a dramatic growth enhancement of *Arabidopsis* under low and moderate light environments (171).

A fast response of stomata to blue light is suggested to decrease stomatal limitation of CO_2 uptake and facilitate CO_2 fixation under sun flecks in the canopy (79). More importantly, the sustained nature of stomatal opening after stimulation by a short period of (blue) light, such as a sun fleck (63), will enhance light-energy capture by providing CO_2 when the leaf encounters successive sun flecks.

Interaction of Blue-Light Signaling with Abscisic Acid

When plants are under drought stress, stomata close in the daytime to minimize water loss by transpiration. ABA induces anion-channel activation with simultaneous membrane depolarization and a subsequent activation of K^+ _{out} channels (11, 144). The activated anion and K^+ _{out} channels allow sustained K^+ salt efflux from guard cells, ultimately resulting in stomatal closure. ABA also stimulates stomatal closure by inhibiting H^+ pumping (48, 157) (**Figure 2**). The pump inhibition is important to maintain membrane depolarization and to reduce ATP consumption by the H^+ -ATPase. ABA inhibits blue-light-dependent phosphorylation of the H^+ -ATPase, and the inhibition may be mediated by H_2O_2 in guard cells (202). ABA produces H_2O_2 in guard cells through NADPH oxidases in the plasma membrane (81, 121, 201, 202), resulting in NO production that stimulates stomatal closure

(22). H_2O_2 also induces an increase through activation of hyperpolarization-activated Ca^{2+} -permeable channels (52, 121). It is possible that the increase in cytosolic Ca^{2+} via H_2O_2 inhibits the H^+ -ATPase, although this seems contradictory if Ca^{2+} is required for blue-light signaling, as mentioned above (74, 90). ABA inhibited blue-light-induced apoplastic acidification in the epidermis by the H^+ -ATPase, and the inhibition was not found in *abi1* and *abi2* mutants of *Arabidopsis* (134). Phototropins could be the target of H_2O_2 , because phototropins have cysteine residues that are essential for their activities (138).

Other Photoreceptors

Phytochrome might be involved in the stomatal movements of several plant species, but the response was quite small (136). Phytochrome was reported as a modulator of the blue-light response of stomata in *Phaseolus vulgaris* seedlings (57). The time required to initiate stomatal opening was shortened by R and lengthened by FR, and the response showed R/FR reversibility. In the orchid *Paphiopedilum*, stomata opened in response to low photon flux density of red light and this opening was reversed by far-red light, suggesting phytochrome involvement (175). Negative results were also reported on phytochrome involvement, e.g., in wheat (71) and *Vicia* (112). Recently, a triple mutation of gene families of phytochrome kinase substrates (*PKS1*, *PKS2*, and *PKS4*) was reported to impair phototropism (85). PKS proteins interacted with phot1 in vivo and in vitro, and PKS proteins were suggested to represent a molecular link between phytochrome and phototropin signaling. However, no data were obtained on the involvement of PKS proteins in the blue-light response of stomata.

The action spectrum of stomatal opening in the UV region was obtained using *Vicia* epidermis (31). The spectrum showed a major peak at 280 nm and a minor peak at 360 nm. The response at 280 nm was three times larger than that at 459 nm. The UV-B-dependent

response was antagonized by green light (30). It is suggested that the response to UV is mediated by a blue-light receptor, and that the energy is directly transferred to the signal molecule from the protein-pigment complex upon absorption of UV. However, *Arabidopsis* mutants of both *npq1* and *phot1 phot2* responded to UV-B (287 nm), suggesting the presence of a separate, unidentified UV-B photoreceptor.

Stomata from the *cry1 cry 2* double mutant exhibited a normal response to blue light, which was enhanced by red light in a manner similar to the response in the wild type, and this result excluded the involvement of cryptochromes in these stomatal responses to light (87). By contrast, a recent detailed investigation indicated that stomata of the *cry1 cry2* double mutant showed a reduced blue-light response, whereas those of CRY1-overexpressing plants revealed a hypersensitive response (92), suggesting the involvement of cryptochromes. In agreement with these findings, stomata in epidermal peels from the *phot1 phot2* double mutant opened slightly in response to relatively strong blue light. Note that in these experiments the blue light stimulus used might have activated chlorophyll-dependent stomatal opening in the epidermis of the *phot1 phot2* double mutant, because the background red light intensity employed would not have sufficed to saturate guard cell photosynthesis. In other cryptochrome-mediated responses, COP1 is implicated as a downstream element. Therefore, in an analysis of stomatal responses in either plants overexpressing *COP1* or plants lacking functional *cop1*, the COP1 protein was implicated as a negative regulator of stomatal opening that functions downstream of both cryptochromes and phototropins. However, stomata in CRY1 overexpressing plants and in *cop1* mutant plants are barely closed (widely open) in darkness. Taken together, these results suggest that cry signaling is likely related to the closure system in stomata, and that COP1 activates the closure system, but is not directly related to phototropin

signaling. This conclusion is also consistent with other reports showing that neither cry1 nor cry2 participates in stomatal opening in response to blue light (111). Crys thus seem to function in a blue-light-independent manner to inhibit stomatal closure and thereby promote stomatal opening.

RED-LIGHT RESPONSE OF STOMATA

The opening response of stomata to red light requires a high light intensity (see **Figure 1**). The red-light response also requires continuous illumination in most plant species, with *Zea mays* being a notable exception (10). The red-light response may be driven partly by accumulation of K⁺ salts (59) and partly by sugar accumulation (112, 174, 176). Potassium accumulation may be driven by the membrane potential, generated by a red-light-activated H⁺ pump in the plasma membrane (112, 150). The pump activation might be brought about by the increase in ATP concentration in the cytosol resulting from photophosphorylation in guard cell chloroplasts (161, 179). However, the pump activation by red light reported in initial patch clamp experiments (150) was not reproduced (135, 177), and confirmation of this finding is needed. Sugars might be produced in guard cells from a combination of starch degradation, photosynthesis, and import from the apoplast (114).

A localized beam of red light applied to individual guard cells did not induce stomatal opening, giving rise to the hypothesis that the red-light response is actually a response to the decrease in *Ci* (**Figure 1**) resulting from red-light-driven mesophyll photosynthesis (131). This result is supported by the recent observation that stomata in albino leaf portions of variegated leaves of *Chlorophytum comosum* do not open by red light (133). The reduction of *Ci* brought about by mesophyll photosynthesis is one of the triggers that cause stomatal opening (**Figure 3**), and it is generally accepted that guard cells sense *Ci* rather than *Ca* (ambient CO₂ concentration) (103). CO₂

modulates cytosolic Ca²⁺ transients in guard cells, thereby stimulating Ca²⁺-induced stomatal closure in a process analogous to that triggered by ABA (144, 187, 192). In addition, guard cells were hyperpolarized in CO₂-free air and depolarized under high CO₂, probably due to the activation of anion channels (18, 53, 131), which results in stomatal closure.

Several lines of evidence indicate that the red-light response is not just an indirect response, mediated by the effects of red light on mesophyll photosynthesis and *Ci*, but also results from a direct response of the guard cells to this light stimulus. First, it is well established that red light can induce stomatal opening in the isolated epidermis. This response depends on guard cell chloroplasts: The response is suppressed by DCMU (112, 148), an inhibitor of PSII, and is not observed in the epidermis of the orchid, *Paphiopedilum*, which has guard cells lacking chlorophyll (193). Experiments in intact leaves also demonstrate that the red-light response is not solely a response to *Ci*: Red light stimulates stomatal opening even when the intracellular concentration of CO₂ is held constant via gas exchange methodology (99).

SYNERGISTIC EFFECT OF BLUE AND RED LIGHT IN STOMATAL OPENING

Stomata in *Arabidopsis* opened rapidly in response to a weak blue light under a background of strong red light, but barely opened without red light (**Figure 1**). Stomatal conductance in leaves illuminated with both red and blue light is typically larger than the sum of the conductances under blue and red light alone, suggesting a synergistic action of stomatal opening in intact leaves. This phenomenon is illustrated in **Figure 1** and has been observed in numerous species (7, 63, 70, 110, 151, 194).

It is likely that both *Ci* and guard cell chloroplasts play roles in the synergistic effect of blue and red light on stomatal opening. That the synergistic effects of blue and red

light could occur in part via a stomatal response to the reduction of C_i induced by mesophyll photosynthesis is suggested by the observation that manipulation of C_i by gas exchange techniques affects the magnitude of the blue-light response. Reducing C_i to a low level increases the magnitude of the blue-light response in several plant species (7, 70, 86). In addition, red-light enhancement of stomatal conductance in response to blue light was found in leaves of the orchid *Paphiopedilum*, which has a chlorophyllous mesophyll but achlorophyllous guard cells, whereas *Paphiopedilum* stomata in isolated epidermal peels showed no red-light response (196). Taken together, these results suggest that mesophyll photosynthesis can indirectly stimulate the blue-light response.

That guard cell chloroplasts also play a role in the synergistic effect of red and blue light is indicated by the fact that this synergism is also found in the isolated epidermis for both stomatal opening (148) and malate formation (109, 110). In addition, in wheat, red light enhanced the magnitude of the blue-light-induced conductance response, even in the presence of CO_2 -free air (70). Such results indicate that there are also C_i -independent aspects to this synergism. We suggest that these C_i -independent aspects could be accounted for by cooperation of blue-light signaling and guard cell chloroplasts with respect to malate

formation. We propose that guard cell chloroplasts are primed to translocate NADH and ATP into the cytosol under red light. These compounds are required for malate synthesis and H^+ pumping, respectively, when blue light is applied (**Figure 4**). Malate is formed by the reduction of oxaloacetate (OAA) by NAD-malate dehydrogenase at the expense of NADH. The large amount of NADH necessary for the reaction can be produced by reverse reactions of NAD^+ -glyceraldehyde 3-phosphate dehydrogenase (NAD^+ -GAPD) in the cytosol using DHAP/GAP as a reductant, which can be supplied by guard cell chloroplasts via the triose phosphate translocator (TPT) under light (**Figure 4**). DHAP/GAP is oxidized to 1,3-bisphosphate glyceric acid (1,3-BPGA), and the ATP is generated by the reverse reaction via phosphoglycerate kinase (PGAK) from 1,3-BPGA, with simultaneous production of 3-PGA (**Figure 4**).

Carbon skeletons for malate formation are derived from starch in the chloroplasts. Blue light induces starch degradation and ultimately provides Triose-P (DHAP/GAP) in the cytosol, and Triose-P is finally converted to PEP. OAA is then produced through PEP carboxylase at the expense of PEP and HCO_3^- (189). It is interesting to note that, in potato, transcript levels of NAD^+ -GAPD, H^+ -ATPase, PEP carboxylase, and inward-rectifying K^+ channel (KST1), all of which are

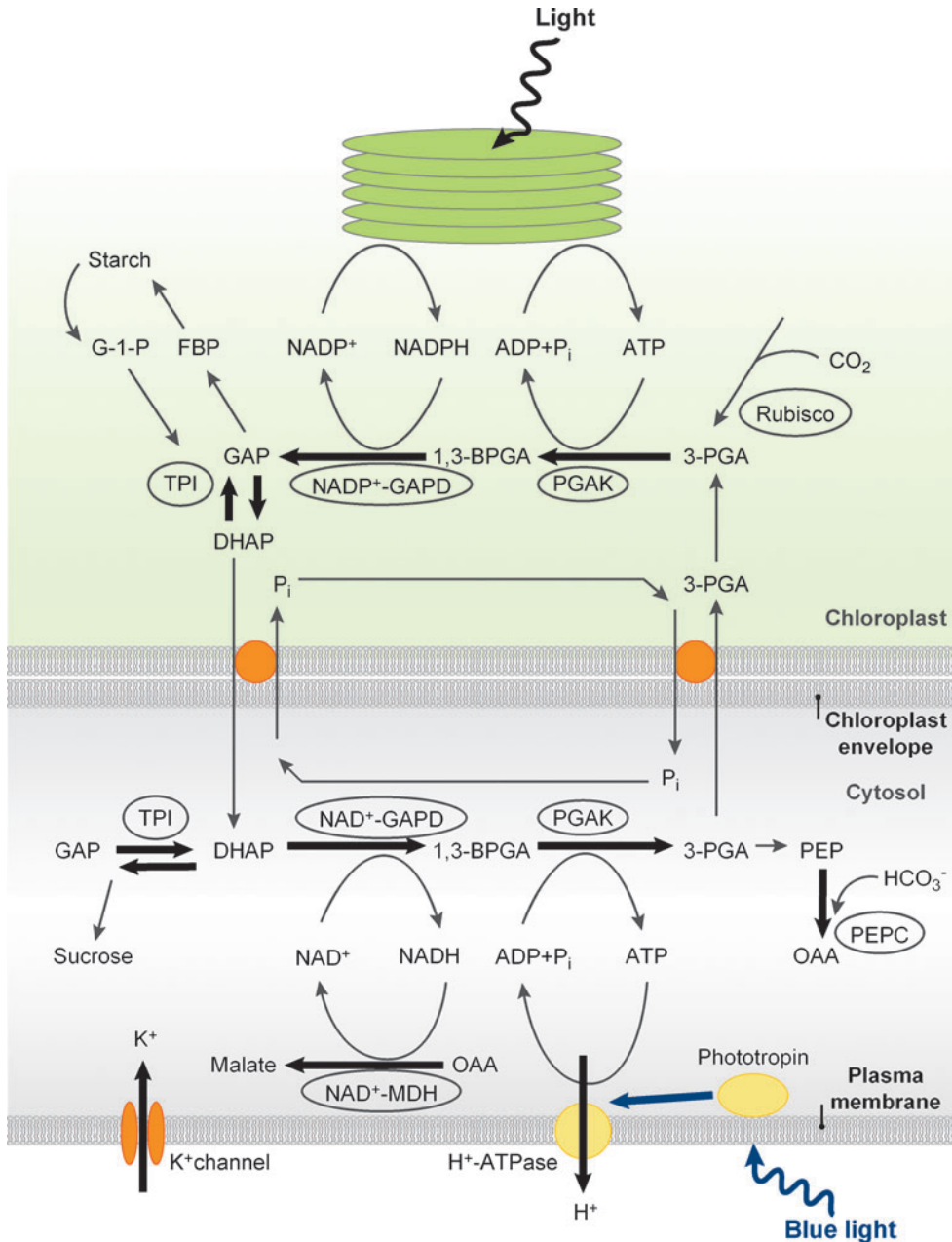
Figure 4

Hypothetical scheme of provision of ATP and NADH from guard cell chloroplasts for blue-light-dependent H^+ pumping in guard cells. Bold black arrows indicate high enzyme activities demonstrated in guard cells. When guard cells are illuminated with blue light, the plasma membrane H^+ -ATPase is activated via phototropin signaling. The activated H^+ -ATPase would consume ATP and induce K^+ uptake through voltage-gated K^+ channels. The accumulated K^+ would promote malate synthesis to compensate the positive charges at the expense of NADH, resulting in the regeneration of ATP and NADH. The resultant 3-PGA would be transported from the cytosol to the chloroplasts, then phosphorylated and reduced to produce DHAP/GAP. DHAP/GAP could be shuttled out from chloroplasts to the cytosol in guard cells, and the phosphorylation and reduction reactions in guard cell chloroplasts would be facilitated under a strong red light. 1,3-BPGA, 1,3-Bisphosphoglycerate; DHAP, Dihydroxyacetone phosphate; FBP, Fructose 1,6-bisphosphate; G-1-P, glucose 1-phosphate; GAP, Glyceraldehyde 3-phosphate; GAPD, Glyceraldehyde-3-phosphate dehydrogenase; MDH, Malate dehydrogenase; OAA, Oxaloacetate; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; PGAK, Phosphoglycerate kinase; 3-PGA, 3-phosphoglycerate; Rubisco, Ribulose-1,5-bisphosphate carboxylase/oxygenase; TPI, Triose-phosphate isomerase.

involved in stomatal opening, were downregulated simultaneously in a guard cell-specific manner under drought (80).

Guard cell chloroplasts have suitable properties to meet this hypothesis (Figure 4). (a) A considerable proportion of reducing equi-

valents and ATP are available for reactions other than photosynthetic CO₂ fixation (153); for example, only 8% of reducing equivalents were used for CO₂ fixation in *Vicia* guard cells (50). (b) The chloroplasts have enzyme activities that favor the accumulation of DHAP;



activities of PGAK, NADP⁺-GAPD, and TPI in guard cell chloroplasts were several to more than 10 times higher than those of mesophyll chloroplasts, but the activity of FBPase in guard cell chloroplasts was roughly half of that in mesophyll chloroplasts. Such accumulation favors the export of DHAP to the cytosol via the phosphate translocator (116, 159). (c) Very high activities of NAD⁺-GAPD and PGAK were found in the guard cell cytosol (159). These enzymes utilize DHAP and catalyze the production of NADH and ATP. (d) Indirect export of ATP, which was generated in guard cell chloroplasts, to the cytosol was suggested by an increase in H⁺ pump activity under red light (179). The H⁺ pump activation was sensitive to DCMU. The ATP production can indicate simultaneous production of NADH in the cytosol.

Therefore, when guard cells are illuminated with blue light under background red light, the activated plasma membrane H⁺-ATPase utilizes ATP for H⁺ pumping, with subsequent K⁺ uptake. This situation requires malate formation, and results in the production of NAD⁺, ADP, 3-PGA, and Pi in the cytosol, and thereby stimulates the export of DHAP/GAP from the chloroplasts (**Figure 4**). A low but significant Rubisco activity in guard cell chloroplasts (23, 127, 153, 200) would generate 3-PGA under red light, and the 3-PGA would act as an intermediate for DHAP production. The enhancement of malate formation is brought about by the activation of the H⁺-ATPase in guard cells (**Figure 4**). In accord with this idea, suppression of PEPC activity through inhibition of H⁺-ATPase was reported (97).

Because blue light likely stimulates the transfer of metabolites between chloroplasts and cytosol (**Figure 4**), it may also affect the energy state of thylakoid membranes. In accord with this idea, two distinct energy states of thylakoid membranes of guard cells were reported. The chlorophyll fluorescence transient in guard cells evoked by blue light had no prominent M peak (98, 155). Such unique kinetics of the fluorescence was transformed

into the standard kinetics of mesophyll cells, with a prominent M peak, when guard cells were excited by green light, which did not activate blue-light signaling (94).

Under CO₂-free conditions, where red-light enhancement of the blue-light response is still observed (70) despite the absence of CO₂ as a substrate for Rubisco, perhaps red light enhances the blue-light stimulation of starch breakdown. Red light promotes starch breakdown under low CO₂ conditions (112), although whether this red-light effect also operates in the presence of blue light has yet to be evaluated.

CONCLUDING REMARKS

Stomatal pores surrounded by a pair of guard cells are important structures and allow uptake of CO₂ for photosynthesis by opening, and reduce the water loss from plants by closing in response to ever-changing environments, thereby extending the land plant growth area. Guard cells are machineries that transduce external and internal signals into the transport of various ions and metabolites across the membranes, and adjust the pore size of stomata. Thus, guard cells are excellent model systems to investigate the mechanisms of perception and transduction of signals, such as light, phytohormones, chemicals, temperature, and humidity, into stomatal movement. In this review, we focused on the light signaling in guard cells and described the properties of the plasma membrane H⁺-ATPase and those of blue-light receptor phototropins. The plasma membrane H⁺-ATPases are distributed in most plant tissues and drive the secondary transport of numerous kinds of inorganic ions, organic acids, and sugars by coupling with tissue- or organ-specific transporters. Because the plasma membrane H⁺-ATPase is activated by blue light in guard cells, the regulatory mechanism of the H⁺-ATPase is elucidated under physiological conditions, which has been difficult in other tissues. Phototropins (phot1 phot2), light receptor-type Ser/Thr protein

kinases, are identified as blue-light receptors, and both phot1 and phot2 function for stomatal opening. Phototropins also regulate divergent responses, including phototropism, chloroplast movement, leaf movement, and leaf flattening. Because the signaling from phototropins to immediate downstream component(s) remains unknown in any of these responses, identification of the kinase substrate or the signaling component in guard cells will provide valuable information. Type 1 protein phosphatase mediates the signaling between phototropins and the plasma membrane H⁺-ATPase in guard cells. Although type 1 protein phosphatase regulates many fundamental processes in animal cells, the role of this phos-

phatase has not been demonstrated in plant cells. Because the type 1 protein phosphatase works together with a number of regulatory subunits, identification of the subunit functioning in blue-light responses is important and will provide new insight into the role of the phosphatase in plant cells. Finally, stomatal opening is supported by metabolic activity unique to guard cells, and malate²⁻ formation is an important process required for the opening. Reducing equivalents and carbon skeletons needed for the malate²⁻ formation are likely provided from guard cell chloroplasts. Guard cell chloroplasts seem to adapt their role to an organ-specific function of stomatal movement.

SUMMARY POINTS

1. Stomatal opening is induced by light and is mediated by two distinct photosystems: blue-light photosystems and chloroplasts. Stomata open in response to a weak blue light and the opening is enhanced by background red light.
2. Blue light activates the plasma membrane H⁺-ATPase via the phosphorylation of the C terminus and increases the inside-negative electrical potential across the plasma membrane in guard cells. The potential drives K⁺ uptake through voltage-gated K⁺ channels, and the accumulated positive K⁺ charges are compensated mainly by malate²⁻ formed in guard cells.
3. Red light induces stomatal opening at high intensity. Red light likely mediates stomatal opening via reduction of the intercellular concentration of CO₂ (*C_i*) by mesophyll photosynthesis, but the role of guard cell chloroplasts in the response could not be excluded.
4. Phototropins (phot1 phot2), light-responsive serine/threonine protein kinases, are identified as blue-light receptors for stomatal opening, and possess flavin mononucleotides as chromophores. Involvement of other photoreceptors in the light-induced opening response of stomata is reported.
5. The signaling between phototropins to the plasma membrane H⁺-ATPase is largely unknown. Results indicate that type 1 protein phosphatase mediates this signaling.
6. Abscisic acid inhibits phototropin-mediated phosphorylation of the plasma membrane H⁺-ATPase via H₂O₂, thereby suppressing stomatal opening.
7. Enhancement of the blue-light response of stomata by red light is brought about by guard cell chloroplast activity. The chloroplasts likely provide ATP for the H⁺-ATPase and NADH for malate²⁻ formation in the cytosol through the translocation of triose phosphate (DHAP/GAP) across the chloroplast envelope.

ACKNOWLEDGMENTS

This work was supported by Grants-in-Aid for Scientific Research (on Priority Areas, no. 17084005 and A, no. 16207003) to K. S., and by grants from the National Science Foundation (MCB02-09694) and the US Department of Agriculture (2006-35100-17254) to S.M.A.

LITERATURE CITED

1. Aitken A. 2002. Functional specificity in 14-3-3 isoform interactions through dimer formation and phosphorylation: Chromosome location of mammalian isoforms and variants. *Plant Mol. Biol.* 50:993–1010
2. Allaway WG, Setterfield G. 1972. Ultrastructural observations on guard cells of *Vicia faba* and *Allium porrum*. *Can. J. Bot.* 50:1405–13
3. Allen GJ, Chu SP, Harrington CL, Schumacher K, Hoffman T, et al. 2001. A defined range of guard cell calcium oscillation parameters encodes stomatal movements. *Nature* 411:1053–57
4. Allen GJ, Chu SP, Schumacher K, Shimazaki CT, Vafeados D, et al. 2000. Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis* det3 mutant. *Science* 289:2338–42
5. Amodeo G, Srivastava A, Zeiger E. 1992. Vanadate inhibits blue light-stimulated swelling of *Vicia* guard cell protoplasts. *Plant Physiol.* 100:1567–70
6. Anderson JA, Huprikar SS, Kochian LV, Lucas WJ, Gaber RF. 1992. Functional expression of a probable *Arabidopsis thaliana* potassium channel in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 89:3736–40
7. Assmann SM. 1988. Enhancement of the stomatal response to blue light by red light, reduced intercellular concentrations of CO₂, and low vapor pressure differences. *Plant Physiol.* 87:226–31
8. Assmann SM. 1993. Signal transduction in guard cells. *Annu. Rev. Cell Biol.* 9:345–75
9. Assmann SM, Grantz DA. 1990. Stomatal response to humidity in sugarcane and soybean: effect of vapor pressure difference on the kinetics of the blue light response. *Plant Cell Environ.* 13:163–69
10. Assmann SM, Lee DM, Markus P. 1992. Rapid stomatal response to red light in *Zea mays*. *Photochem. Photobiol.* 56:685–89
11. Assmann SM, Shimazaki K. 1999. The multisensory guard cell: Stomatal responses to blue light and abscisic acid. *Plant Physiol.* 119:809–15
12. Assmann SM, Simoncini L, Schroeder JL. 1985. Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba* L. *Nature* 318:285–87
13. Assmann SM, Zeiger E. 1987. Guard cell bioenergetics. In *Stomatal Function*, ed. E Zeiger, G Farquhar, I Cowan, pp. 163–94. Stanford, CA: Stanford Univ. Press
14. Babourina O, Newman I, Shabara S. 2002. Blue light-induced kinetics of H⁺ and Ca²⁺ fluxes in etiolated wild-type and phototropin-mutant *Arabidopsis* seedlings. *Proc. Natl. Acad. Sci. USA* 99:2433–38
15. Bachmann M, Huber JL, Athwal GS, Wu K, Ferl RJ, Huber SC. 1996. 14-3-3 proteins associate with the regulatory phosphorylation site of spinach leaf nitrate reductase in an isoform-specific manner and reduce dephosphorylation of Ser-543 by endogenous protein phosphatases. *FEBS Lett.* 398:26–30
16. Baum G, Long JC, Jenkins GI, Trewavas AJ. 1999. Stimulation of the blue light phototropic receptor NPH1 causes a transient increase in cytosolic Ca²⁺. *Proc. Natl. Acad. Sci. USA* 96:13554–59

17. Blatt MR. 2000. Cellular signaling and volume control in stomatal movements in plants. *Annu. Rev. Cell Dev. Biol.* 16:221–41
18. Brearley J, Venis MA, Blatt MR. 1997. The effect of elevated CO₂ concentrations on K⁺ and anion channels of *Vicia faba* L. guard cells. *Planta* 203:145–54
19. Briggs WR. 2005. Phototropin overview. In *Light Sensing in Plants*, ed. M Wada, K Shimazaki, M Iino, pp. 139–46. Tokyo: The Bot. Soc. Jpn./Springer-Verlag
20. Briggs WR, Beck CF, Cashmore AR, Christie JM, Hughes J, et al. 2001. The phototropin family of photoreceptors. *Plant Cell* 13:993–97
21. Briggs WR, Christie JM. 2002. Phototropins 1 and 2: versatile plant blue-light receptors. *Trends Plant Sci.* 7:204–10
22. Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ. 2006. ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H₂O₂ synthesis. *Plant J.* 45:113–22
23. Cardon ZG, Berry J. 1992. Effects of O₂ and CO₂ concentration on the steady-state fluorescence yield of single guard cell pairs in intact leaf discs of *Tradescantia albiflora*. *Plant Physiol.* 99:1238–44
24. Christie JM, Reymond P, Powell GK, Bernasconi P, Raibekas AA, et al. 1998. *Arabidopsis* NPH1: A flavoprotein with the properties of a photoreceptor for phototropism. *Science* 282:1698–701
25. Christie JM, Salomon M, Nozue K, Wada M, Briggs WR. 1999. LOV (light, oxygen, or voltage) domains of the blue light photoreceptor phototropin (nph1): Binding sites for the chromophore flavin mononucleotide. *Proc. Natl. Acad. Sci. USA* 96:8779–83
26. DeWitt ND, Sussman MR. 1995. Immunocytological localization of an epitope-tagged plasma membrane proton pump (H⁺-ATPase) in phloem companion cells. *Plant Cell* 7:2053–67
27. Ditttrich P, Raschke K. 1977. Uptake and metabolism of carbohydrates by epidermal tissue. *Planta* 134:83–90
28. Doi M, Shigenaga A, Emi T, Kinoshita T, Shimazaki K. 2004. A transgene encoding a blue-light receptor, phot1, restores blue-light responses in the *Arabidopsis phot1 phot2* double mutant. *J. Exp. Bot.* 55:517–23
29. Eckert M, Kaldenhoff R. 2000. Light-induced stomatal movement of selected *Arabidopsis thaliana* mutants. *J. Exp. Bot.* 51:1435–42
30. Eisinger W, Bogomolni RA, Taiz L. 2003. Interaction between a blue-green reversible photoreceptor and separate UV-B receptor in stomatal guard cells. *Am. J. Bot.* 90:1560–66
31. Eisinger W, Swartz TE, Bogomolni RA, Taiz L. 2000. The UV action spectrum for stomatal opening in broad bean. *Plant Physiol.* 122:99–105
32. Emi T, Kinoshita T, Sakamoto K, Mineyuki Y, Shimazaki K. 2005. Isolation of a protein interacting with Vfphot1a in guard cells of *Vicia faba*. *Plant Physiol.* 138:1615–26
33. Emi T, Kinoshita T, Shimazaki K. 2001. Specific binding of vf14-3-3a isoform to the plasma membrane H⁺-ATPase in response to blue light and fusicoccin in guard cells of broad bean. *Plant Physiol.* 125:1115–25
34. Fairley-Grenot K, Assmann SM. 1992. Whole cell K⁺ current across the plasma membrane of guard cells from a grass: *Zea mays*. *Planta* 186:282–93
35. Fan LM, Zhao Z, Assmann SM. 2004. Guard cells: a dynamic signaling model. *Curr. Opin. Plant Biol.* 7:537–46
36. Ferl RJ. 1996. 14-3-3 proteins and signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:49–73
37. Fischer RA. 1968. Stomatal opening: role of potassium uptake by guard cells. *Science* 160:784–85

38. Folta KM, Lieg EJ, Durham T, Spalding EP. 2003. Primary inhibition of hypocotyl growth and phototropism depend differentially on phototropin-mediated increases in cytoplasmic calcium induced by blue light. *Plant Physiol.* 133:1464–70
39. Frechilla S, Talbott LD, Bogomolni RA, Zeiger E. 2000. Reversal of blue light-stimulated stomatal opening by green light. *Plant Cell Physiol.* 41:171–76
40. Frechilla S, Zhu J, Talbott LD, Zeiger E. 1999. Stomata from *npq1*, a zeaxanthin-less *Arabidopsis* mutant, lack a specific response to blue light. *Plant Cell Physiol.* 40:949–54
41. Fridlyand LE, Backhausen JE, Scheibe R. 1998. Flux control of the malate valve in leaf cells. *Arch. Biochem. Biophys.* 349:290–98
42. Fujino M. 1967. Role of ATP and ATPase in stomatal movements. *Sci. Bull. Fac. Educ. Nagasaki Univ.* 18:1–47
43. Gao XQ, Li CG, Wei PC, Zhang XY, Chen J, Wang XC. 2005. The dynamic changes of tonoplasts in guard cells are important for stomatal movement in *Vicia faba*. *Plant Physiol.* 139:1207–16
44. Gautier H, Vavasseur A, Gans P, Lascève G. 1991. Relationship between respiration and photosynthesis in guard cell and mesophyll cell protoplasts of *Commelina communis* L. *Plant Physiol.* 95:636–41
45. Gautier H, Vavasseur A, Lascève G, Boudet AM. 1992. Redox processes in the blue light response of guard cell protoplasts of *Commelina communis* L. *Plant Physiol.* 98:34–38
46. Gepstein S, Jacobs M, Taiz L. 1982. Inhibition of stomatal opening in *Vicia faba* epidermal tissue by vanadate and abscisic acid. *Plant Sci. Lett.* 28:63–72
47. Goh CH, 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.* 32:623–30
48. Goh CH, Kinoshita T, Oku T, Shimazaki K. 1996. Inhibition of blue light dependent H⁺ pumping by abscisic acid in *Vicia* guard-cell protoplasts. *Plant Physiol.* 111:433–40
49. Gotow K, Tanaka K, Kondo N, Kobayashi K, Syono K. 1985. Light activation of NADP-malate dehydrogenase in guard cell protoplasts from *Vicia faba* L. *Plant Physiol.* 79:829–32
50. Gotow K, Taylor S, Zeiger E. 1988. Photosynthetic carbon fixation in guard cell protoplasts of *Vicia faba* L.: Evidence from radiolabel experiments. *Plant Physiol.* 86:700–5
51. Guo FQ, Young J, Crawford NM. 2003. The nitrate transporter AtNRT1.1 (CHL1) functions in stomatal opening and contributes to drought susceptibility in *Arabidopsis*. *Plant Cell* 15:107–17
52. Hamilton DW, Hills A, Köhler B, Blatt MR. 2000. Ca²⁺ channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid. *Proc. Natl. Acad. Sci. USA* 97:4967–72
53. Hanstein SM, Felle HH. 2002. CO₂-triggered chloride release from guard cells in intact fava bean leaves. Kinetics of the onset of stomatal closure. *Plant Physiol.* 130:940–50
54. Harada A, Sakai T, Okada K. 2003. phot1 and phot2 mediate blue light-induced transient increases in cytosolic Ca²⁺ differently in *Arabidopsis* leaves. *Proc. Natl. Acad. Sci. USA* 100:8583–88
55. Hentzen AF, Smart LB, Wimmers LE, Fang HH, Schroeder JI, Bennett AB. 1996. Two plasma membrane H⁺-ATPase genes expressed in guard cells of *Vicia faba* are also expressed throughout the plant. *Plant Cell Physiol.* 37:650–59
56. Hetherington AM. 2001. Guard cell signaling. *Cell* 107:711–14
57. Holmes MG, Klein WH. 1985. Evidence for phytochrome involvement in light-mediated stomatal movements in *Phaseolus vulgaris* L. *Planta* 166:348–53

58. Houlne G, Boutry M. 1994. Identification of an *Arabidopsis thaliana* gene encoding a plasma membrane H⁺-ATPase whose expression is restricted to anther tissue. *Plant J.* 5:311–17
59. Hsiao TC, Allway WG, Evans LT. 1973. Action spectra for guard cell Rb⁺ uptake and stomatal opening. *Plant Physiol.* 51:82–88
60. Huala E, Oeller PW, Liscum E, Han IS, Larsen E, Briggs WR. 1997. *Arabidopsis* NPH1: a protein kinase with a putative redox-sensing domain. *Science* 278:2120–23
61. Huang K, Beck CF. 2003. Phototropin is the blue-light receptor that controls multiple steps in the sexual life cycle of the green algae *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* 100:6269–74
62. Huber ST, MacKintosh C, Kaiser WM. 2002. Metabolic enzymes as targets for 14-3-3 proteins. *Plant Mol. Biol.* 50:1053–63
63. Iino M, Ogawa T, Zeiger E. 1985. Kinetic properties of the blue-light response of stomata. *Proc. Natl. Acad. Sci. USA* 82:8019–23
64. Imamura S. 1943. Untersuchungen über den Mechanismus der turgorschwankung der Spaltöffnungsschließzellen. *Jpn. J. Bot.* 12:251–346
65. Inada S, Ohgishi M, Mayama T, Okada K, Sakai T. 2004. RPT2 is a signal transducer involved in phototropic response and stomatal opening by association with phototropin 1 in *Arabidopsis thaliana*. *Plant Cell* 16:887–96
66. Inoue S, Kinoshita T, Shimazaki K. 2005. Possible involvement of phototropins in leaf movement of kidney bean in response to blue light. *Plant Physiol.* 138:1994–2004
67. Jarillo JA, Ahmad M, Cashmore AR. 1998. NPL1: A second member of the NPH1 serine/threonine protein kinase family of *Arabidopsis*. *Plant Physiol.* 117:719
68. Jarillo JA, Gabrys H, Capel J, Alonso JM, Ecker JR, Cashmore AR. 2001. Phototropin-related NPL1 controls chloroplast relocation induced by blue light. *Nature* 410:952–54
69. Kagawa T, Sakai T, Suetsugu N, Oikawa K, Ishiguro S, et al. 2001. *Arabidopsis* NPL1: A phototropin homolog controlling the chloroplast high-light avoidance response. *Science* 291:2138–41
70. Karlsson PE. 1986. Blue light regulation of stomata in wheat seedlings. I. Influence of red background illumination and initial conductance level. *Physiol. Plant.* 66:202–6
71. Karlsson PE. 1986. Blue light regulation of stomata in wheat seedlings. II. Action spectrum and search for action dichroism. *Physiol. Plant.* 66:207–10
72. Kinoshita T, Doi M, Suetsugu N, Kagawa T, Wada M, Shimazaki K. 2001. phot1 and phot2 mediate blue light regulation of stomatal opening. *Nature* 414:656–60
73. Kinoshita T, Emi T, Tominaga M, Sakamoto K, Shigenaga A, et al. 2003. Blue-light- and phosphorylation-dependent binding of a 14-3-3 protein to phototropins in stomatal guard cells of broad bean. *Plant Physiol.* 133:1453–63
74. Kinoshita T, Nishimura M, Shimazaki K. 1995. Cytosolic concentration of Ca²⁺ regulates the plasma membrane H⁺-ATPase in guard cells of fava bean. *Plant Cell* 7:1333–42
75. Kinoshita T, Shimazaki K. 1997. Involvement of calyculin A- and okadaic acid-sensitive protein phosphatase in the blue light response of stomatal guard cells. *Plant Cell Physiol.* 38:1281–85
76. Kinoshita T, Shimazaki K. 1999. Blue light activates the plasma membrane H⁺-ATPase by phosphorylation of the C-terminus in stomatal guard cells. *EMBO J.* 18:5548–58
77. Kinoshita T, Shimazaki K. 2001. Analysis of the phosphorylation level in guard-cell plasma membrane H⁺-ATPase in response to fusicoccin. *Plant Cell Physiol.* 42:424–32
78. Kinoshita T, Shimazaki K. 2002. Biochemical evidence for the requirement of 14-3-3 protein binding in activation of the guard-cell plasma membrane H⁺-ATPase by blue light. *Plant Cell Physiol.* 43:1359–65

79. Kirschbaum MUF, Pearcy RW. 1988. Gas exchange analysis of the relative importance of stomatal and biochemical factors in photosynthetic induction in *Alocasia macrorrhiza*. *Plant Physiol.* 86:782–85
80. Kopka J, Provart NJ, Müller-Röber B. 1997. Potato guard cells respond to drying soil by a complex change in the expression of genes related to carbon metabolism and turgor regulation. *Plant J.* 11:871–82
81. Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, et al. 2003. NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. *EMBO J.* 22:2623–33
82. Kwak JM, Murata Y, Baizabal-Aguirre VM, Merrill J, Wang M, et al. 2001. Dominant negative guard cell K⁺ channel mutants reduce inward-rectifying K⁺ currents and light-induced stomatal opening in *Arabidopsis*. *Plant Physiol.* 127:473–85
83. Lahav M, Abu-Abied M, Belasov E, Schwartz A, Sadot E. 2004. Microtubules of guard cells are light sensitive. *Plant Cell Physiol.* 45:573–82
84. Langer K, Levchenko V, Fromm J, Geiger D, Steinmeyer R, et al. 2004. The poplar K⁺ channel KPT1 is associated with K⁺ uptake during stomatal opening and bud development. *Plant J.* 37:828–38
85. Lariguet P, Schepens I, Hodgson D, Pedmale UV, Trevisan M, et al. 2006. PHYTOCHROME KINASE SUBSTRATE1 is a phototropin1 binding protein required for phototropism. *Proc. Natl. Acad. Sci. USA* 103:10134–39
86. Lascève G, Gautier H, Jappe J, Vavasseur A. 1993. Modulation of the blue light response of stomata of *Commelina communis* by CO₂. *Physiol. Plant.* 88:453–59
87. Lascève G, Leymarie J, Olney MA, Liscum E, Christie JM, et al. 1999. *Arabidopsis* contains at least four independent blue-light-activated signal transduction pathways. *Plant Physiol.* 120:605–14
88. Lascève G, Leymarie J, Vavasseur A. 1997. Alterations in light-induced stomatal opening in a starch-deficient mutant of *Arabidopsis thaliana* L. deficient in chloroplast phosphoglucomutase activity. *Plant Cell Environ.* 20:350–58
89. Lee DM, Assmann SM. 1992. Stomatal responses to light in the facultative Crassulacean acid metabolism species, *Portulacaria afra*. *Physiol. Plant.* 85:35–42
90. Lino B, Baizabal-Aguirre VM, Vara LEG. 1998. The plasma-membrane H⁺-ATPase from beet root is inhibited by a calcium-dependent phosphorylation. *Planta* 204:352–59
91. Lu P, Outlaw WHJ, Smith BG, Freed GA. 1997. A new mechanism for the regulation of stomatal aperture size in intact leaves (accumulation of mesophyll-derived sucrose in the guard-cell wall of *Vicia faba*). *Plant Physiol.* 114:109–18
92. Mao J, Zhang YC, Sang Y, Li QH, Yang HQ. 2005. A role for *Arabidopsis* cryptochromes and COP1 in the regulation of stomatal opening. *Proc. Natl. Acad. Sci. USA* 102:12270–75
93. Mawson BT. 1993. Modulation of photosynthesis and respiration in guard and mesophyll cell protoplasts by oxygen concentration. *Plant Cell Environ.* 16:207–14
94. Mawson BT, Zeiger E. 1991. Blue light-modulation of chlorophyll *a* fluorescence transients in guard cell chloroplasts. *Plant Physiol.* 96:753–60
95. McAinsh MR, Hetherington AM. 1998. Encoding specificity in Ca²⁺ signaling systems. *Trends Plant Sci.* 3:32–36
96. McAinsh MR, Webb AAR, Taylor JE, Hetherington AM. 1995. Stimulus-induced oscillation in guard cell cytosolic free calcium. *Plant Cell* 7:1207–19
97. Meinhard M, Schnabl H. 2001. Fusicocin- and light-induced activation and in vivo phosphorylation of phosphoenolpyruvate carboxylase in *Vicia* guard cell protoplasts. *Plant Sci.* 160:635–46

98. Melis A, Zeiger E. 1982. Chlorophyll *a* fluorescence transients in mesophyll and guard cells. *Plant Physiol.* 69:642–47
99. Messinger SM, Buckley TN, Mott KA. 2006. Evidence for involvement of photosynthetic processes in the stomatal responses to CO₂. *Plant Physiol.* 140:771–78
100. Meyer S, Lauterbach C, Niedermeier M, Barth I, Sjolund RD, Sauer N. 2004. Wounding enhances expression of AT5G03330, a sucrose transporter from *Arabidopsis* sieve elements and sink tissues. *Plant Physiol.* 134:684–93
101. Morrison D. 1994. 14-3-3: Modulators of signaling proteins. *Science* 266:56–57
102. Motchoulski A, Liscum E. 1999. *Arabidopsis* NPH3: A NPH1 photoreceptor-interacting protein essential for phototropism. *Science* 286:961–64
103. Mott KA. 1988. Do stomata respond to CO₂ concentrations other than intercellular? *Plant Physiol.* 86:200–3
104. Müller-Röber B, Ellenberg J, Provart N, Willmitzer L, Busch H, et al. 1995. Cloning and electrophysiological analysis of KST1, an inward rectifying K⁺ channel expressed in potato guard cells. *EMBO J.* 14:2409–16
105. Muslin AJ, Tanner JM, Allen PM, Shaw AS. 1996. Interaction of 14-3-3 protein with signaling proteins is mediated by the recognition of phosphoserine. *Cell* 84:889–97
106. Nakajima N, Saji H, Aono M, Kondo N. 1995. Isolation of cDNA for a plasma membrane H⁺-ATPase from guard cells of *Vicia faba* L. *Plant Cell Physiol.* 36:919–24
107. Nakamura RL, McKendree WL, Hirsch RE, Sedbrook JC, Gaber RF, Sussman MR. 1995. Expression of an *Arabidopsis* potassium channel gene in guard-cells. *Plant Physiol.* 109:371–74
108. Niyogi KK, Grossman AR, Bjorkman O. 1998. *Arabidopsis* mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. *Plant Cell* 10:1121–34
109. Ogawa T. 1981. Blue light response of stomata with starch-containing (*Vicia faba*) and starch-deficient (*Allium cepa*) guard cells under background illumination with red light. *Plant Sci. Lett.* 22:103–8
110. Ogawa T, Ishikawa H, Shimada K, Shibata K. 1978. Synergistic action of red and blue light and action spectra for malate formation in guard cells of *Vicia faba* L. *Planta* 142:61–65
111. Ohgishi M, Saji K, Okada K, Sakai T. 2004. Functional analysis of each blue light receptor, cry1, cry2, phot1, and phot2, by using combinational multiple mutants in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 101:2223–28
112. Olsen RL, Pratt RB, Gump P, Kemper A, Tallman G. 2002. Red light activates a chloroplast-dependent ion uptake mechanism for stomatal opening under reduced CO₂ concentrations in *Vicia* spp. *N. Phytol.* 153:497–508
113. Onodera A, Kong SG, Doi M, Shimazaki K, Christie J, et al. 2005. Phototropin from *Chlamydomonas reinhardtii* is functional in *Arabidopsis thaliana*. *Plant Cell Physiol.* 46:367–74
114. Outlaw WHJ, Manchester J. 1979. Guard cell starch concentration quantitatively related to the stomatal aperture. *Plant Physiol.* 64:79–82
115. Outlaw WHJ, Manchester J, DiCamelli CA, Randall DD, Rapp B, Veith GM. 1979. Photosynthetic carbon reduction pathway is absent in chloroplasts of *Vicia faba* guard cells. *Proc. Natl. Acad. Sci. USA* 76:6371–75
116. Overlach S, Diekmann W, Raschke K. 1993. Phosphate translocator of isolated guard-cell chloroplasts from *Pisum sativum* L. transports glucose-6-phosphate. *Plant Physiol.* 101:1201–7

117. Palevitz BA, O’Kane DJ, Korb RE, Raikhel NV. 1981. The vacuole system in stomatal cells of *Allium*. Vacuole movements and changes in morphology in differentiating cells as revealed by epifluorescence, video and electron microscopy. *Protoplasma* 109:23–35
118. Palmer JM, Short TM, Briggs WR. 1993. Correlation of blue light-induced phosphorylation to phototropism in *Zea mays* L. *Plant Physiol.* 102:1219–25
119. Palmgren MG. 2001. Plant plasma membrane H⁺-ATPase: power houses for nutrient uptake. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52:817–45
120. Parvathi K, Raghavendra AS. 1995. Bioenergetic processes in guard cells related to stomatal function. *Physiol. Plant.* 93:146–54
121. Pei ZM, Murata Y, Benning G, Thomine S, Klüsener B, et al. 2000. Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. *Nature* 406:731–34
122. Pei ZM, Ward JM, Harper JF, Schroeder JI. 1996. A novel chloride channel in *Vicia faba* guard cell vacuoles activated by the serine/threonine kinase, CDPK. *EMBO J.* 15:6564–74
123. Philippar K, Ivashikina N, Ache P, Christian M, Lüthen H, et al. 2004. Auxin activates *KAT1* and *KAT2*, two K⁺-channel genes expressed in seedlings of *Arabidopsis thaliana*. *Plant J.* 37:815–27
124. Poffenroth M, Green DB, Tallman G. 1992. Sugar concentrations in guard cells of *Vicia faba* illuminated with red or blue light: analysis by high performance liquid chromatography. *Plant Physiol.* 98:1460–71
125. Raghavendra AS. 1990. Blue light effects on stomata are mediated by the guard cell plasma membrane redox system distinct from the proton translocating ATPase. *Plant Cell Environ.* 13:105–10
126. Raschke K, Humble GD. 1973. No uptake of anions required by opening stomata of *Vicia faba*: guard cells release hydrogen ions. *Planta* 115:47–57
127. Reckmann U, Sheibe R, Raschke K. 1990. Rubisco activity in guard cells compared with the solute requirement for stomatal opening. *Plant Physiol.* 92:246–53
128. Reddy AR, Das VSR. 1986. Stomatal movements and sucrose uptake by guard cell protoplasts of *Commelina benghalensis* L. *Plant Cell Physiol.* 27:1565–70
129. Ritte G, Raschke K. 2003. Metabolite export of isolated guard cell chloroplasts of *Vicia faba*. *N. Phytol.* 159:195–202
130. Ritte G, Rosenfeld J, Rohrig K, Raschke K. 1999. Rates of sugar uptake by guard cell protoplasts of *Pisum sativum* L. related to the solute requirement for stomatal opening. *Plant Physiol.* 121:647–56
131. Roelfsema MRG, Hanstein S, Felle HH, Hedrich R. 2002. CO₂ provides an intermediate link in the red light response of guard cells. *Plant J.* 32:65–75
132. Roelfsema MRG, Hedrich R. 2005. In the light of stomatal opening: New insights into ‘the Watergate’. *N. Phytol.* 167:665–91
133. Roelfsema MRG, Konrad KR, Marten H, Psaras GK, Hartung W, Hedrich R. 2006. Guard cells in albino leaf patches do not respond to photosynthetically active radiation, but are sensitive to blue light, CO₂, and abscisic acid. *Plant Cell Environ.* 29:1595–605
134. Roelfsema MRG, Staal M, Prins ABA. 1998. Blue light-induced apoplastic acidification of *Arabidopsis thaliana* guard cells: Inhibition by ABA is mediated through protein phosphatases. *Physiol. Plant.* 103:466–74
135. Roelfsema MRG, Steinmeyer R, Staal M, Hedrich R. 2001. Single guard cell recordings in intact plants: light-induced hyperpolarization of the plasma membrane. *Plant J.* 26:1–13
136. Roth-Bejerano N, Itai C. 1987. Phytochrome involvement in stomatal movement in *Pisum sativum*, *Vicia faba*, and *Pelargonium*, sp. *Physiol. Plant.* 70:85–89

137. Sakamoto K, Briggs WR. 2002. Cellular and subcellular localization of phototropin 1. *Plant Cell* 14:1723–35
138. Salomon M, Christie JM, Knieb E, Lempert U, Briggs WR. 2000. Photochemical and mutational analysis of the FMN-binding domains of the plant blue light receptor, phototropin. *Biochemistry* 39:9401–10
139. Salomon M, Knieb E, von Zepelin T, Rüdiger W. 2003. Mapping of low- and high-fluence autophosphorylation sites in phototropin 1. *Biochemistry* 42:4217–25
140. Sanders D, Pelloux J, Brownlee C, Harper JF. 2002. Calcium at the crossroads of signaling. *Plant Cell* 14:S401–17
141. Schachtman DP, Schroeder JI, Lucas WJ, Anderson JA, Gaber RF. 1992. Expression of an inward-rectifying potassium channel by the *Arabidopsis* KAT1 cDNA. *Science* 258:1654–58
142. Scheibe R, Reckmann U, Hedrich R, Raschke K. 1990. Malate dehydrogenase in guard cells of *Pisum sativum*. *Plant Physiol.* 93:1358–64
143. Schroeder JI. 1988. K⁺ transport properties of K⁺ channels in the plasma membrane of *Vicia faba* guard cells. *J. Gen. Physiol.* 92:667–83
144. Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D. 2001. Guard cell signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52:627–58
145. Schroeder JI, Hagiwara S. 1989. Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells. *Nature* 338:427–30
146. Schroeder JI, Raschke K, Neher E. 1987. Voltage dependence of K⁺ channels in guard-cell protoplasts. *Proc. Natl. Acad. Sci. USA* 84:4108–12
147. Schwartz A. 1985. Role of calcium and EGTA on stomatal movements in *Commelina communis*. *Plant Physiol.* 79:1003–5
148. Schwartz A, Zeiger E. 1984. Metabolic energy for stomatal opening: Roles of photophosphorylation and oxidative phosphorylation. *Planta* 161:129–36
149. Sentenac H, Bonneaud N, Minet M, Lacroute F, Salmon JM, et al. 1992. Cloning and expression in yeast of a plant potassium-ion transport-system. *Science* 256:663–65
150. Serrano EE, Zeiger E, Hagiwara S. 1988. Red light stimulates an electrogenic proton pump in *Vicia* guard cell protoplasts. *Proc. Natl. Acad. Sci. USA* 85:436–40
151. Sharkey TD, Ogawa T. 1987. Stomatal responses to light. In *Stomatal Function*, ed. E Zeiger, G Farquhar, I Cowan, pp. 195–208. Stanford, CA: Stanford Univ. Press
152. Sharkey TD, Raschke K. 1981. Separation and measurement of direct and indirect effects of light on stomata. *Plant Physiol.* 68:33–40
153. Shimazaki K. 1989. Ribulosebiphosphate carboxylase activity and photosynthetic O₂ evolution rate in *Vicia* guard cell protoplasts. *Plant Physiol.* 91:459–63
154. Shimazaki K, Goh CH, Kinoshita T. 1999. Involvement of intracellular Ca²⁺ in blue light-dependent proton pumping in guard cell protoplasts from *Vicia faba*. *Physiol. Plant.* 105:554–61
155. Shimazaki K, Gotow K, Kondo N. 1982. Photosynthetic properties of guard cell protoplasts from *Vicia faba* L. *Plant Cell Physiol.* 23:871–79
156. Shimazaki K, Gotow K, Sakaki T, Kondo N. 1983. High respiratory activity of guard cell protoplasts from *Vicia faba* L. *Plant Cell Physiol.* 23:871–79
157. Shimazaki K, Iino M, Zeiger E. 1986. Blue light-dependent proton extrusion by guard-cell protoplasts of *Vicia faba*. *Nature* 319:324–26
158. Shimazaki K, Kinoshita T, Nishimura M. 1992. Involvement of calmodulin and calmodulin-dependent myosin light chain kinase in blue light-dependent H⁺ pumping by guard cell protoplasts from *Vicia faba* L. *Plant Physiol.* 99:1416–21
159. Shimazaki K, Terada J, Tanaka K, Kondo N. 1989. Calvin-Benson cycle enzymes in guard-cell protoplasts from *Vicia faba* L. *Plant Physiol.* 90:1057–64

160. Shimazaki K, Tominaga M, Shigenaga A. 1997. Inhibition of the stomatal blue light response by verapamil at high concentration. *Plant Cell Physiol.* 38:747–50
161. Shimazaki K, Zeiger E. 1985. Cyclic and noncyclic photophosphorylation in isolated guard cell chloroplasts from *Vicia faba* L. *Plant Physiol.* 78:211–14
162. Shope JC, DeWald DB, Mott KA. 2003. Changes in surface area of intact guard cells are correlated with membrane internalization. *Plant Physiol.* 133:1314–21
163. Short TW, Briggs WR. 1994. The transduction of blue light signals in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45:143–71
164. Smith AM, Zeeman SC, Smith SM. 2005. Strach degradation. *Annu. Rev. Plant Biol.* 56:73–98
165. Sondergaard TE, Schulz A, Palmgren MG. 2004. Energization of transport processes in plants. Roles of the plasma membrane H⁺-ATPase. *Plant Physiol.* 136:2475–82
166. Stadler R, Büttner M, Ache P, Hedrich R, Ivashikina N, et al. 2003. Diurnal and light-regulated expression of *AtSTP1* in guard cells of *Arabidopsis*. *Plant Physiol.* 133:528–37
167. Stoelzle S, Kagawa T, Wada M, Hedrich R, Dietrich P. 2003. Blue light activates calcium-permeable channels in *Arabidopsis* mesophyll cells via the phototropin signaling pathway. *Proc. Natl. Acad. Sci. USA* 100:1456–61
168. Sussman MR. 1994. Molecular analysis of proteins in the plant plasma membrane. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44:253–61
169. Svennelid F, Olsson A, Piotrowski M, Rosenquist M, Ottman C, et al. 1999. Phosphorylation of Thr-948 at the C terminus of the plasma membrane H⁺-ATPase creates a binding site for the regulatory 14-3-3 protein. *Plant Cell* 11:2379–91
170. Szyroki A, Ivashikina N, Dietrich P, Roelfsema MRG, Ache P, et al. 2001. KAT1 is not essential for stomatal opening. *Proc. Natl. Acad. Sci. USA* 98:2917–21
171. Takemiya A, Inoue S, Doi M, Kinoshita T, Shimazaki K. 2005. Phototropins promote plant growth in response to blue light in low light environment. *Plant Cell* 17:1120–27
172. Takemiya A, Kinoshita T, Asanuma M, Shimazaki K. 2006. Protein phosphatase 1 positively regulates stomatal opening in response to blue light in *Vicia faba*. *Proc. Natl. Acad. Sci. USA* 103:13549–54
173. Talbott LD, Hammad JW, Harn LC, Nguyen VH, Patel J, Zeiger E. 2006. Reversal by green light of blue light-stimulated stomatal opening in intact, attached leaves of *Arabidopsis* operates only in the potassium-dependent, morning phase of movement. *Plant Cell Physiol.* 47:332–39
174. Talbott LD, Zeiger E. 1998. The role of sucrose in guard cell osmoregulation. *J. Exp. Bot.* 49:329–37
175. Talbott LD, Zhu JX, Han SW, Zeiger E. 2002. Phytochrome and blue light-mediated stomatal opening in the orchid, *Paphiopedilum*. *Plant Cell Physiol.* 43:639–46
176. Tallman G, Zeiger E. 1988. Light quality and osmoregulation in *Vicia* guard cells. Evidence for involvement of three metabolic pathways. *Plant Physiol.* 88:887–95
177. Taylor AR, Assmann SM. 2001. Apparent absence of a redox requirement for blue light activation of pump current in broad bean guard cells. *Plant Physiol.* 125:329–38
178. Tlalka M, Fricker M. 1999. The role of calcium in blue-light-dependent chloroplast movement in *Lemna trisulca* L. *Plant J.* 20:461–73
179. Tominaga M, Kinoshita T, Shimazaki K. 2001. Guard-cell chloroplasts provide ATP required for H⁺ pumping in the plasma membrane and stomatal opening. *Plant Cell Physiol.* 42:795–802
180. Ueno K, Kinoshita T, Inoue S, Emi T, Shimazaki K. 2005. Biochemical characterization of the plasma membrane H⁺-ATPase activation in guard-cell protoplasts of *Arabidopsis thaliana* in response to blue light. *Plant Cell Physiol.* 46:955–63

181. Vani T, Raghavendra AS. 1989. Tetrazolium reduction by guard cells in abaxial epidermis of *Vicia faba*. Blue light stimulation of a plasmalemma redox system. *Plant Physiol.* 90:59–62
182. 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.* 105:1263–68
183. Vavasseur A, Raghavendra AS. 2005. Guard cell metabolism and CO₂ sensing. *N. Phytol.* 165:665–82
184. Vèry AA, Gaymard F, Bosseux C, Sentenac H, Thibaud JB. 1995. Expression of a cloned plant K⁺ channel in *Xenopus* oocytes: analysis of macroscopic currents. *Plant J.* 7:321–32
185. Vitart V, Baxter I, Doerner P, Harper JF. 2001. Evidence for a role in growth and salt resistance of a plasma membrane H⁺-ATPase in the root endodermis. *Plant J.* 27:191–201
186. Ward JM, Pei ZM, Schroeder JI. 1995. Roles of ion channels in initiation of signal transduction in higher plants. *Plant Cell* 7:833–44
187. Webb AAR, McAinsh MR, Mansfield TA, Hetherington AM. 1996. Carbon dioxide induces increases in guard cell cytosolic free calcium. *Plant J.* 9:297–304
188. Wille AC, Lucas WJ. 1984. Ultrastructural and histochemical studies on guard cells. *Planta* 160:129–42
189. Willmer CM, Fricker MD. 1996. *Stomata*. London, UK: Chapman & Hall. 375 pp. 2nd ed.
190. Wu WH, Assmann SM. 1995. Is ATP required for K⁺ channel activation in *Vicia* guard cells? *Plant Physiol.* 107:101–9
191. Yamashita T. 1952. Influences of potassium supply upon various properties and movement of guard cell. *Sieboldia Acta Biol.* 1:51–70
192. Young JJ, Mehta S, Israelsson M, Godoski J, Grill E, Schroeder JI. 2006. CO₂ signaling in guard cells: Calcium sensitivity response modulation, a Ca²⁺-independent phase, and CO₂ insensitivity of the *gca2* mutant. *Proc. Natl. Acad. Sci. USA* 103:7506–11
193. Zeiger E. 1983. The biology of stomatal guard cells. *Annu. Rev. Plant Physiol.* 34:441–75
194. Zeiger E. 1984. Blue light and stomatal function. In *Blue Light Effects in Biological Systems*, ed. H Senger, pp. 484–94. New York/Tokyo: Springer-Verlag
195. Zeiger E, Armond P, Melis A. 1981. Fluorescence properties of guard cell chloroplasts. *Plant Physiol.* 67:17–20
196. Zeiger E, Assmann SM, Meidner H. 1983. The photobiology of *Paphiopedilum* stomata—opening under blue but not red-light. *Photochem. Photobiol.* 38:627–30
197. Zeiger E, Hepler PK. 1977. Light and stomatal function: Blue light stimulates swelling of guard cell protoplasts. *Science* 196:887–89
198. Zeiger E, Talbott LD, Frechilla S, Srivastava A, Zhu J. 2002. The guard cell chloroplast: a perspective for the twenty-first century. *N. Phytol.* 153:415–24
199. Zeiger E, Zhu J. 1998. Role of zeaxanthin in blue light photoreception and the modulation of light-CO₂ interactions in guard cells. *J. Exp. Bot.* 49:433–42
200. Zemel E, Gepstein S. 1985. Immunological evidence for the presence of ribulose biphosphate carboxylase in guard cell chloroplasts. *Plant Physiol.* 78:586–90
201. Zhang X, Miao YC, An GY, Zhou Y, Shangguan ZP, et al. 2001. K⁺ channels inhibited by hydrogen peroxide mediate abscisic acid signaling in guard cells. *Cell Res.* 11:195–202
202. Zhang X, Wang H, Takemiya A, Song CP, Kinoshita T, Shimazaki K. 2004. Inhibition of blue light-dependent H⁺ pumping by abscisic acid through hydrogen peroxide-induced dephosphorylation of the plasma membrane H⁺-ATPase in guard cell protoplasts. *Plant Physiol.* 136:4150–58



Contents

Frontispiece	
<i>Diter von Wettstein</i>	xii
From Analysis of Mutants to Genetic Engineering	
<i>Diter von Wettstein</i>	1
Phototropin Blue-Light Receptors	
<i>John M. Christie</i>	21
Nutrient Sensing and Signaling: NPKS	
<i>Daniel P. Schachtman and Ryoung Shin</i>	47
Hydrogenases and Hydrogen Photoproduction in Oxygenic Photosynthetic Organisms	
<i>Maria L. Ghirardi, Matthew C. Posewitz, Pin-Ching Maness, Alexandra Dubini, Jianping Yu, and Michael Seibert</i>	71
Hidden Branches: Developments in Root System Architecture	
<i>Karen S. Osmont, Richard Sibout, and Christian S. Hardtke</i>	93
Leaf Senescence	
<i>Pyung Ok Lim, Hyo Jung Kim, and Hong Gil Nam</i>	115
The Biology of Arabinogalactan Proteins	
<i>Georg J. Seifert and Keith Roberts</i>	137
Stomatal Development	
<i>Dominique C. Bergmann and Fred D. Sack</i>	163
Gibberellin Receptor and Its Role in Gibberellin Signaling in Plants	
<i>Miyako Ueguchi-Tanaka, Masatoshi Nakajima, Ashikari Motoyuki, and Makoto Matsuoka</i>	183
Cyclic Electron Transport Around Photosystem I: Genetic Approaches	
<i>Toshiharu Shikanai</i>	199
Light Regulation of Stomatal Movement	
<i>Ken-ichiro Shimazaki, Michio Doi, Sarah M. Assmann, and Toshinori Kinoshita</i>	219

The Plant Heterotrimeric G-Protein Complex <i>Brenda R.S. Temple and Alan M. Jones</i>	249
Alternative Splicing of Pre-Messenger RNAs in Plants in the Genomic Era <i>Anireddy S.N. Reddy</i>	267
The Production of Unusual Fatty Acids in Transgenic Plants <i>Johnathan A. Napier</i>	295
Tetrapyrrole Biosynthesis in Higher Plants <i>Ryouichi Tanaka and Ayumi Tanaka</i>	321
Plant ATP-Binding Cassette Transporters <i>Philip A. Rea</i>	347
Genetic and Epigenetic Mechanisms for Gene Expression and Phenotypic Variation in Plant Polyploids <i>Z. Jeffrey Chen</i>	377
Tracheary Element Differentiation <i>Simon Turner, Patrick Gallois, and David Brown</i>	407
<i>Populus</i> : A Model System for Plant Biology <i>Stefan Jansson and Carl J. Douglas</i>	435
Oxidative Modifications to Cellular Components in Plants <i>Ian M. Møller, Poul Erik Jensen, and Andreas Hansson</i>	459
Indexes	
Cumulative Index of Contributing Authors, Volumes 48–58	483
Cumulative Index of Chapter Titles, Volumes 48–58	488
Errata	
An online log of corrections to <i>Annual Review of Plant Biology</i> chapters (if any, 1997 to the present) may be found at http://plant.annualreviews.org/	