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INTERACTIONS BETWEEN A BLUE-GREEN REVERSIBLE PHOTORECEPTOR AND A SEPARATE UV-B RECEPTOR IN STOMATAL GUARD CELLS[1]

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Stomatal opening exhibits two main peaks of activity in the visible range—a red peak, mediated by photosynthesis, and a blue peak, mediated by one or more blue light (BL) photoreceptors. In addition, a pronounced peak in the UV-B region has been characterized, as has a smaller UV-A peak. The BL-induced stomatal opening can be reversed by green light (GL). Here we report that UV-B-induced opening is also antagonized by GL. To determine whether UV-B is being absorbed by the BL photoreceptor or by a separate UV-B receptor, the UV-B responses of two different *Arabidopsis* mutants, *npq1* and *phot1/phot2,* were tested. Both putative BL-photoreceptor mutants exhibited normal stomatal opening in response to UV-B, consistent with the existence of a separate UV-B photoreceptor. Moreover, GL failed to antagonize UV-B-induced stomatal opening in the *phot1/phot2* double mutant and only partially antagonized UV-B opening in *npq1*. Thus, both phot1 and phot 2, as well as zeaxanthin, are required for the normal GL inhibition of UV-B. A model for a photoreceptor network that regulates stomatal opening is presented. Unlike the situation in guard cells, the UV-B bending response of *Arabidopsis* hypocotyls during phototropism appears to be mediated by phototropins.

Key words: *Arabidopsis*; guard cell; photoreceptor; phototropin; phototropism; stomate; *Vicia faba*; zeaxanthin.

Stomatal opening is one of many physiological responses of plants regulated by blue light (BL). Recently, progress has been made in identifying several genes encoding putative photoreceptors for BL responses, including *CRY1* and *CRY2,* involved in such diverse processes as hypocotyl elongation (Ahmad and Cashmore, 1993; Cashmore et al., 1999), anthocyanin synthesis (Ahmad et al., 1995; Wade et al., 2001), and flowering (Guo et al., 1998), and *PHOT1* and *PHOT2,* which encode phototropins, the BL photoreceptors involved in phototropism (Christie et al., 1998; Briggs and Christie, 2002) and chloroplast movement (Jarillo et al., 2001; Kagawa et al., 2001).

The *CRY* and *PHOT* genes encode flavoproteins, consistent with the presence of a peak in the UV-A in the action spectra for their responses. *Trans*-carotenoids, which make up the bulk of the carotenoids in the chloroplast, do not absorb UV-A. Thus, a peak in the UV-A region of the action spectrum has been considered diagnostic for flavoproteins. Recently, we reported the ultraviolet action spectrum for stomatal opening in *Vicia faba* (Eisinger et al., 2000). A small peak of activity at around 360 nm was observed, as was a much larger peak at around 280 nm, consistent with a flavoprotein BL photoreceptor in guard cells. This prediction was confirmed by Kinoshita et al. (2001), who showed that both *PHOT1* and *PHOT2* were required for BL-induced stomatal opening.

In contrast, Zeiger and his colleagues have proposed the carotenoid zeaxanthin as the BL photoreceptor of guard cells (Zeiger, 2000). A key finding in support of the model is that stomata of *npq1,* a mutant of *Arabidopsis* blocked at the zeaxanthin de-epoxidase step of zeaxanthin biosynthesis, fail to open in response to blue light, although they show normal red light-induced opening (Frechilla et al., 1999). More recently, Zeiger and colleagues have determined that green light reverses the effects of blue light on stomatal opening (Frechilla et al., 2000). By analogy with phytochrome, blue-green photoreversibility of stomatal opening is consistent with a model in which the zeaxanthin chromophore undergoes a *trans*-to-*cis* isomerization within the chloroplast, which results in the transmission of a signal to the cytosol, where the response occurs (Zeiger, 2000). *Cis*-carotenoids, unlike *trans*-carotenoids, have an absorption peak in the UV-A region (Molnar and Szabolcs, 1993). However, according to the model, absorption of light by *cis*-zeaxanthin should convert the pigment to the inactive *trans* form, inhibiting stomatal opening, not promoting it. Thus the zeaxanthin model is not in accord with the presence of a small peak of activity in the UV-A region of the action spectrum, but rather supports the participation of phototropins 1 and 2 in UV-A-induced stomatal opening.

The significance of the large peak in the UV-B region of the spectrum is unclear. Treatment with UV-B in the presence of saturating BL failed to cause any additional stomatal opening. Therefore, we initially proposed that the UV-B and BL responses could be mediated by the same protein-pigment molecule, with BL being absorbed by the chromophore and UV-B absorbed by the protein (Eisinger et al., 2000).

In this report, we follow up our previous observations by investigating the interactions between ultraviolet (UV-B and UV-A) and visible (blue and green) wavelengths in stomatal opening. We also characterize the UV and visible light responses of two xanthophyll-cycle mutants of *Arabidopsis*, *npq1* and *aba1*. The *aba1* mutant is blocked in the synthesis of the enzyme zeaxanthin epoxidase, the enzyme that converts zeaxanthin to violaxanthin, and is characterized by an excess of zeaxanthin (Duckham et al., 1991; Rock and Zeevaart, 1991). The responses of the *phot1/phot2* double mutant to both UV-B and GL were also determined. Finally, we examined the phototropic bending responses of the *phot1/phot2* mutants in response to UV-B. All of the data collected thus far are con-

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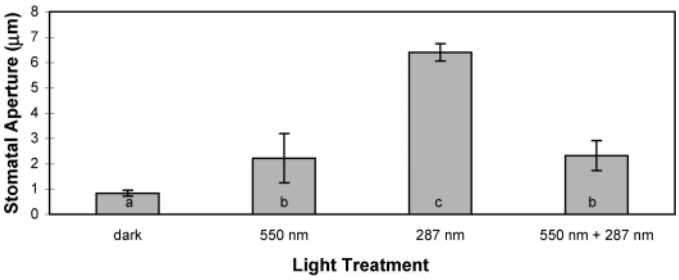

Fig. 1. Stomatal opening in epidermal peels of *Vicia faba* in response to green (550 nm) and UV-B (287 nm) light treatments. Means ± 1 SE labeled with different letters are significantly different according to a one-way analysis of variance ($P < 0.05$).

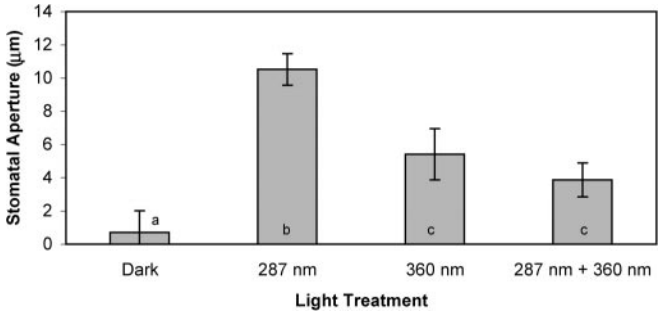

Fig. 3. Stomatal opening in epidermal peels of *Vicia faba* in response to UV-B (287 nm) and UV-A (360 nm) light treatments. Means ± 1 SE labeled with different letters are significantly different according to a one-way analysis of variance ($P < 0.05$).

sistent with a model based on a complex photoreceptor network regulating stomatal opening. The UV-B response in guard cells is mediated by a separate UV-B receptor. In contrast, the UV-B response of phototropism appears to be mediated primarily by phot1 and phot2.

MATERIALS AND METHODS

Five-week-old, greenhouse-grown *Vicia faba* plants were placed in the dark for 1 h to induce stomatal closure. All experiments were carried out in a dark room. Epidermal peels were prepared under dim red light (0.2 μmol · m^{-2} · s^{-1}) and floated on a medium containing 0.1 mol/L KCl and 0.1 mmol/L $CaCl_2$ in a petri dish. For a given experiment, all epidermal peels were taken from the same leaf, while each treatment was given to three peels from different regions of the same leaf.

Arabidopsis (mutants and their *Col* and *Ler* background) plants were grown in a plant growth chamber at 22°C with a 10-h photoperiod for about a month. Only rosette leaves were used and collected from plants in the vegetative growth stage. Because the leaves were often too small to prepare epidermal peels, stomatal apertures were measured from whole-leaf mounts under glass slides. Whole-leaf mounts gave faster and more reproducible results than epidermal peels.

The UV-light source was a 75-W xenon arc lamp. Specific wavelengths were selected using a Bausch & Lomb (Rochester, New York, USA) high intensity monochromator. For UV experiments, stray visible light was excluded from the monochromater beam using a Corning 7-54 quartz filter (Kopp/Corning, Pittsburgh, Pennsylvania, USA) that transmits only light in the 250–410 nm range. The monochromator was positioned on a shelf above the bench and the beam was directed downwards by means of a front-surface mirror. Fluence rates were determined using a PIN-8 photodiode (United Detector Technologies, Baltimore, Maryland, USA) with extended UV range, which was previously spectrally calibrated against a Kettering 68 (Milton Roy, Florida, USA) thermopile in the 220–700 nm range. The Kettering radiometer calibration is traceable to NBS standard lamps. The calibration was confirmed within 10% using the actinochrome chemical actinometer (Brauer et al., 1983).

After 1 h of light treatment, the peels were viewed in a Zeiss fluorescence light microscope (Oberkochen, Germany) using a 40× objective and digitally recorded using a Panasonic model WV 850 infrared-sensitive ($\lambda \geq 800$ nm) video camera (New York, New York, USA) and a Packard-Bell PBTV4 video capture card (Sacramento, California, USA). The digitized images were later viewed with Adobe Photoshop (San Jose, California, USA) software, and stomatal apertures were measured using the ''Information Palette'' feature of that program. A minimum of 50 stomata were measured per light treatment, and each experiment was repeated a minimum of three times.

In experiments involving the combination of two wavelengths, the primary light source was a halogen bulb (Phillips 20 MR 16, Koninklijke Philips Electronics, Somerset, New Jersey, USA) with a Tempax wide band hot mirror filter (Schott Glaswerke, Mainz, Germany). The fluence at tissue level was approximately 500 μmol · m^{-2} · s^{-1}. Corning glass filters plus hot mirrors or narrow band interference filters were used to provide specific wavelength ranges: 650 nm (630–750 nm); 550 nm (545–555 nm), and 450 nm (400–490 nm). The secondary light source was the xenon arc lamp with monochromator (12 nm bandwidth) described earlier set at 284 nm or 360 nm and providing approximately 0.18 μmol · m^{-2} · s^{-1} at tissue level. After an hour of illumination, peels were photographed as described and stomatal apertures were analyzed using Photoshop.

For phototropism experiments, seedlings were grown in potting mixture for 5–6 d in the dark, placed ~2 m from the monochromator on the bench top. The fluence rate at tissue level was approximately 1 μmol · m^{-2} · s^{-1} for blue light experiments and 0.1 μmol · m^{-2} · s^{-1} for UV-B experiments. A Corning 7-54 filter transmitting only wavelengths between 250 and 410 nm excluded stray visible light in UV-B experiments. Seedlings were irradiated with uni-

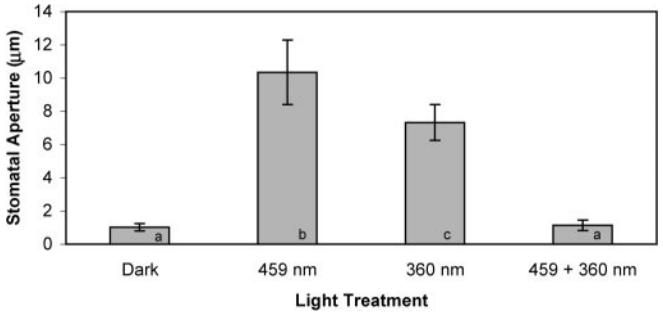

Fig. 2. Stomatal opening in epidermal peels of *Vicia faba* in response to blue (459 nm) and UV-A (360 nm) light treatments. Means ± 1 SE labeled with different letters are significantly different according to a one-way analysis of variance ($P < 0.05$).

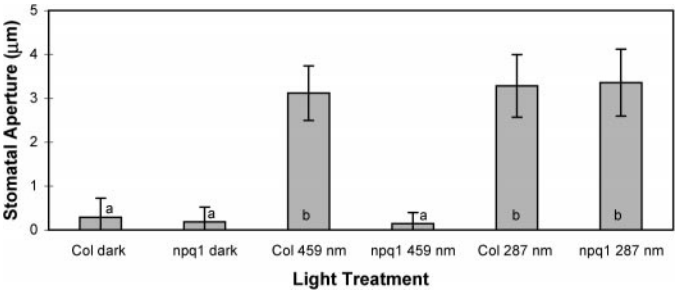

Fig. 4. Stomatal opening of zeaxanthin-deficient mutant, *npq1*, and Columbia (Col) wild-type *Arabidopsis* in response to blue light (459 nm) and UV-B (287 nm) treatments. Means ± 1 SE labeled with different letters are significantly different according to a one-way analysis of variance ($P < 0.05$).

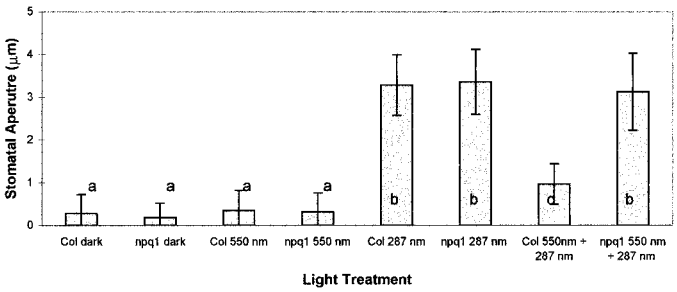

Fig. 5. Stomatal opening of *npq1* and Columbia (Col) wild-type *Arabidopsis* in response to green (550 nm) and UV-B (287 nm) light treatments. Means ± 1 SE labeled with different letters are significantly different according to a one-way analysis of variance ($P < 0.05$).

lateral blue light (450 nm) or UV-B (280 nm) for 6–24 h and then photographed with a digital camera.

RESULTS

Preliminary experiments with *Vicia faba* confirmed the results of Frechilla et al. (2000) that GL antagonized the effects of BL in stomatal opening (data not shown). We therefore tested whether GL could block the effects of UV-B on stomatal opening. The results are shown in Fig. 1. Green light alone induced a small amount of opening of *Vicia faba* stomata. However, when given in combination with UV-B, GL strongly antagonized the stimulatory effects of UV-B on stomatal opening. The ability of GL to antagonize UV-B-induced as well as BL-induced stomatal opening suggests that the fundamental mechanisms of BL-induced and UV-B-induced stomatal opening are the same.

As shown in Fig. 2, UV-A (360 nm) alone is able to induce an intermediate amount of opening, ~60% of the opening induced by blue light, consistent with the previously established action spectrum (Eisinger et al., 2000). However, when given in combination with BL, UV-A blocked BL-induced opening to the level of the dark control. Thus, UV-A is at least as effective as GL in antagonizing BL-induced opening.

The ability of UV-A to inhibit UV-B-induced opening was also tested. As shown in Fig. 3, a similar pattern was observed. Irradiation with UV-A alone caused some stomatal opening, and also inhibited opening induced by UV-B. However, UV-A was less effective in blocking UV-B-induced stomatal opening (Fig. 3).

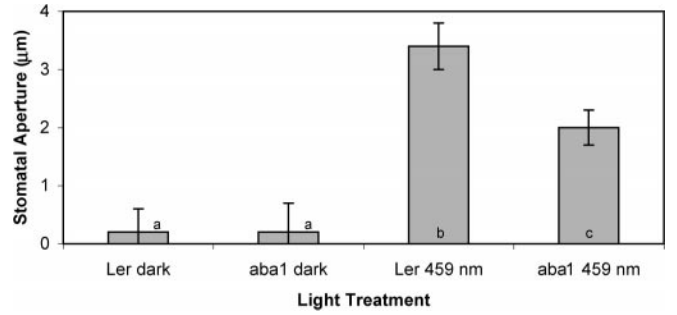

Fig. 6. Stomatal opening of ABA-deficient, zeaxanthin-overproducing mutant, *aba1,* and *Landsberg erecta* (Ler) wild-type *Arabidopsis* in response to blue light (459 nm) treatment. Means ± 1 SE labeled with different letters are significantly different according to a one-way analysis of variance ($P < 0.05$).

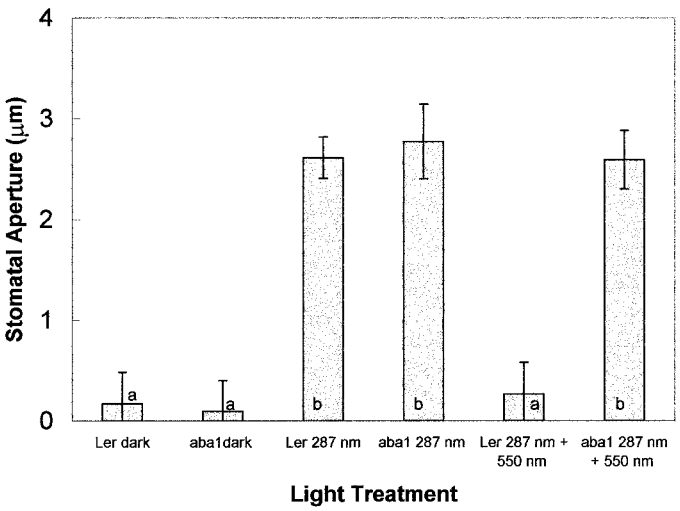

Fig. 7. Stomatal opening of *aba1* and *Landsberg erecta* (Ler) wild-type *Arabidopsis* in response to UV-B (287 nm) and green light (550 nm) treatments. Means ± 1 SE labeled with different letters are significantly different according to a one-way analysis of variance ($P < 0.05$).

In studies with the zeaxanthin-deficient mutant, *npq1,* we were able to confirm that, under the conditions employed, the stomatal opening response to BL (0.1 μmol · m^{-2} · s^{-1}) was absent (Fig. 4). Although unable to respond to dim BL, *npq1* nevertheless had a normal opening response to UV-B (Fig. 4).

Green light is an effective antagonist of UV-B-induced stomatal opening in wild-type plants (see Fig. 1). As shown in Fig. 5, although the stomata of *npq1* opened in response to UV-B, the effect could not be blocked by green light, suggesting a role for zeaxanthin in the responses to both BL and GL.

As a control for *npq1,* we also characterized the stomatal responses of *aba1* to dim blue light (0.1 μmol · m^{-2} · s^{-1}). If bulk zeaxanthin functions as the BL photoreceptor, *aba1,* which accumulates zeaxanthin, would be expected to be more sensitive to BL than the wild type. However, the *aba1* mutants

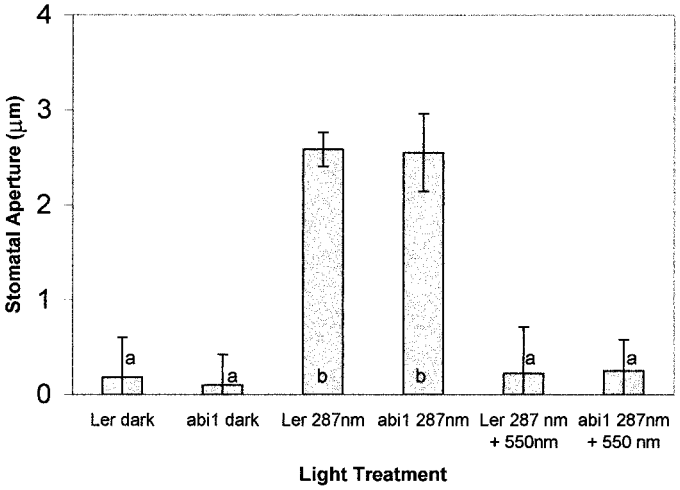

Fig. 8. Stomatal opening of *abi1* and *Landsberg erecta* (Ler) wild-type *Arabidopsis* in response to UV-B (287 nm) and green light (550 nm) treatments. Means ± 1 SE labeled with different letters are significantly different according to a one-way analysis of variance ($P < 0.05$).

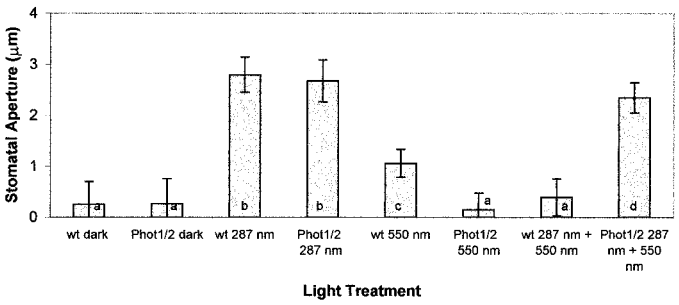

Fig. 9. Stomatal opening of the *phot1/phot2* double mutant and wild-type *Arabidopsis* in response to UV-B (287 nm) and green light (550 nm) treatments. Bars show means ± 1 SE.

were less sensitive to blue light than the wild type rather than more sensitive (Fig. 6). This result rules out bulk zeaxanthin as the BL photoreceptor in stomatal opening.

Although their response to dim BL was reduced, *aba1* stomata opened normally in response to UV-B irradiation (Fig. 7). As with *npq1*, UV-B-induced opening could not be blocked by GL in the *aba1* mutant (Fig. 7). Thus, the responses of both xanthophyll-cycle mutants were qualitatively similar. To test whether the absence of ABA in the *aba1* mutant might be affecting the stomatal opening response to light, we also examined the ABA-insensitive mutant *abi1* and observed that it responded normally to UV-B and showed normal GL inhibition of UV-B-induced opening (Fig. 8). Thus, the failure of *aba1* to respond to GL cannot be due to the absence of an ABA response.

We also examined the UV-B and GL responses of the *phot1/phot2* double mutant stomata, which are unable to respond to BL. As shown in Fig. 9, stomatal guard cells of *phot1/phot2* double mutants respond normally to UV-B irradiation. However, GL fails to antagonize UV-B-induced opening in the *phot1/phot2* mutant, as in the case of *npq1*.

Finally, the persistence of normal UV-B stomatal opening in the *phot1/phot2* mutant raised the question of whether UV-B-induced phototropism would also be normal in the absence of the two phototropins. As shown in Fig. 10A, the *phot1/phot2* mutant hypocotyls failed to bend in response to BL, even after 24 h of exposure to dim blue light. Preliminary experiments showed that the wild type begins to bend in response to UV-B light by 3 h, at which time the mutant fails to respond. Wild-type *Arabidopsis* seedlings exhibited a pronounced bending response after 24-h exposure to UV-B irradiation (Fig. 10B). The response of the *phot1/phot2* mutants to UV-B irradiation was quite variable among the seedlings. This bending response was drastically reduced in the *phot1/phot2* mutant when compared with wild type, although it was not entirely eliminated. Thus, phot1 and phot2 appear to be the primary UV-B receptors in phototropism. The residual, highly variable bending response to UV-B irradiation over extended periods of time appears to be by some other mechanism, based perhaps on direct auxin inactivation, and is probably not physiologically significant.

DISCUSSION

The ultraviolet action spectrum for stomatal opening exhibits a large peak in the UV-B region of the spectrum in addition to a small peak in the UV-A (Eisinger et al., 2000). Although the effects of BL and UV-B were not found to be additive (Eisinger et al., 2000), the experiments described in the present study provide strong evidence that the UV-B response is mediated by a separate UV-B receptor. The two main candidates for the BL photoreceptor are the carotenoid zeaxanthin and the flavoproteins, phot1 and phot2.

One of the main arguments used to support the zeaxanthin hypothesis has been the correlation between bulk zeaxanthin levels and the BL response. Pretreating guard cells with increasing fluences of red light enhanced both total zeaxanthin content and the response to a subsequent BL treatment (Fre-

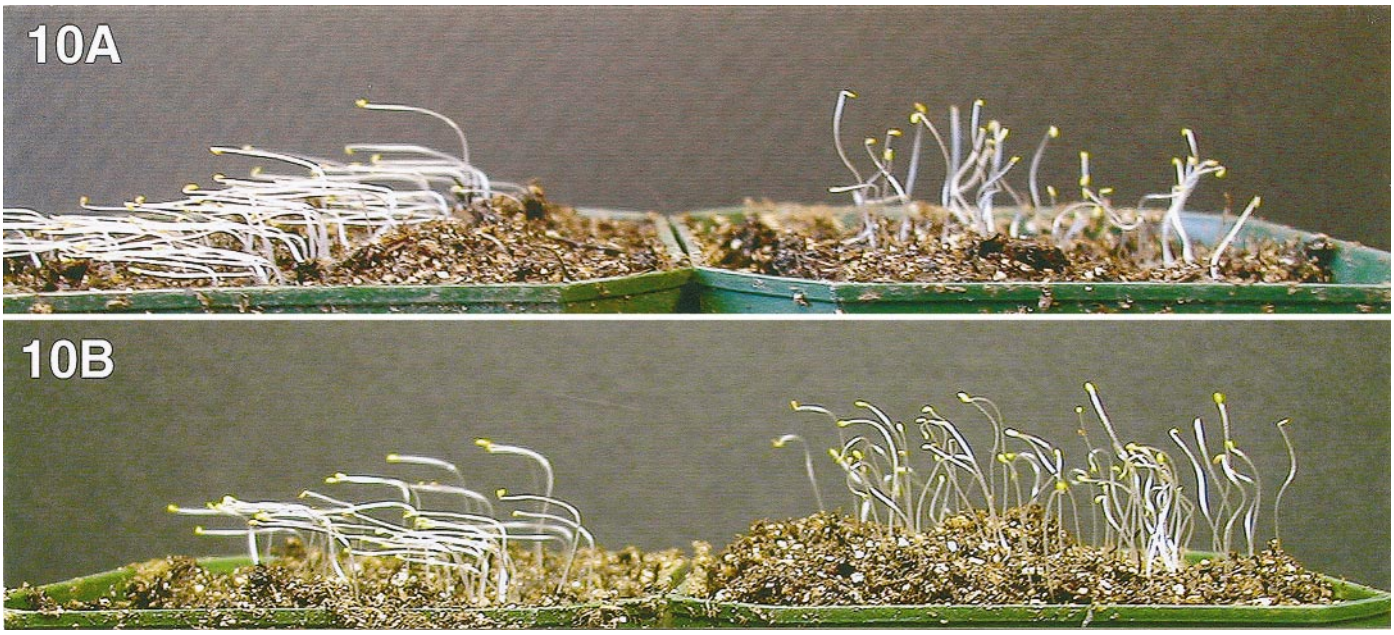

Fig. 10. Responses of wild type (left) and *phot1/phot2* double mutant (right) to 24 h of unilateral blue light (A) and unilateral UV-B irradiation (B). The *phot1/phot2* double mutant seedlings fail to respond to blue light and show only a small residual response to UV-B.

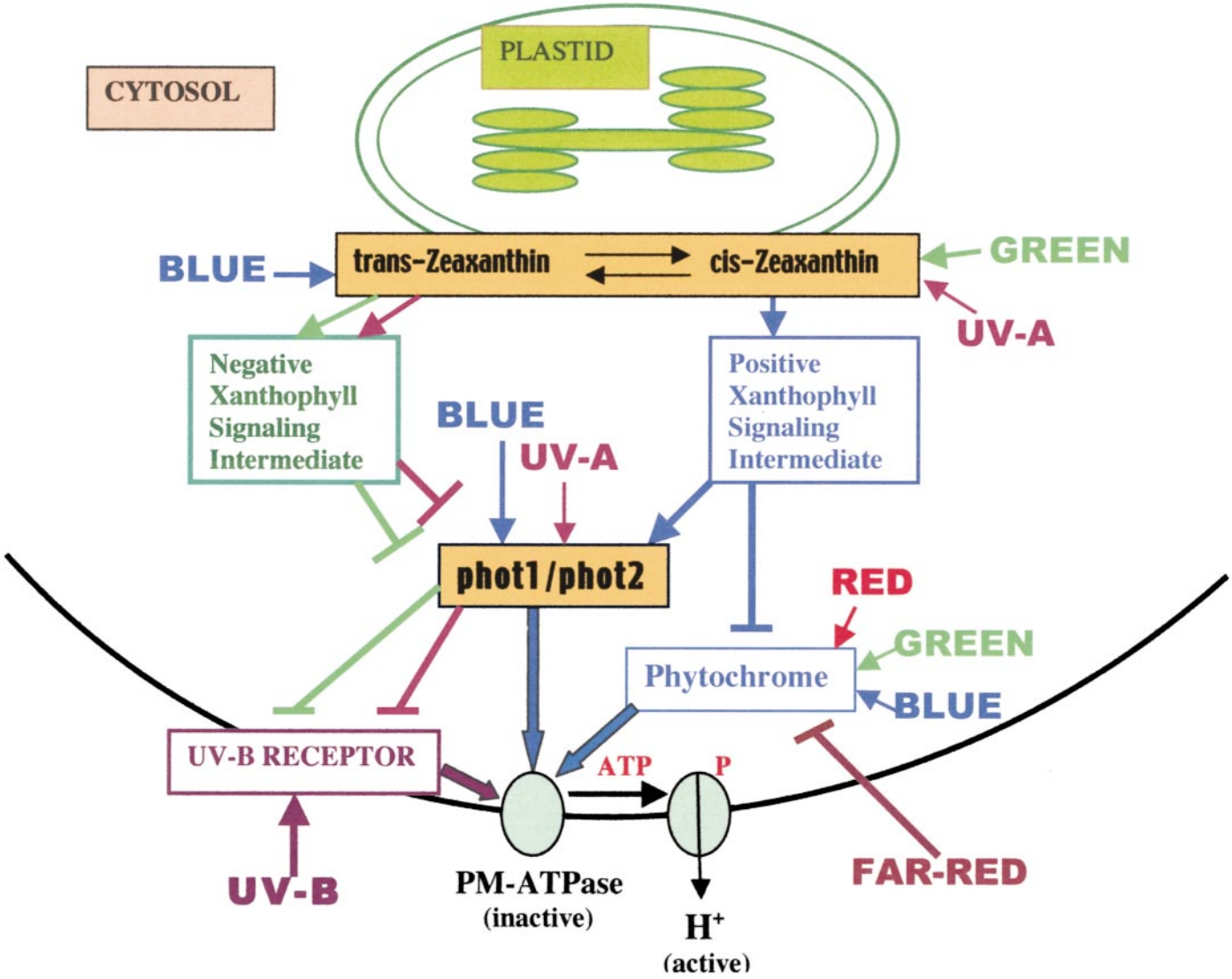

Fig. 11. Model for the regulation of stomatal opening by a photoreceptor network, including a functioning xanthophyll cycle, phot1 and phot2, phytochrome, and a UV-B receptor. See Discussion.

chilla et al., 1999). Conversely, treatment with 3 mmol/L DTT (dithiothreitol) inhibited zeaxanthin synthesis and also inhibited BL-dependent stomatal opening (Frechilla et al., 1999). However, our finding that the *aba1* mutant, which accumulates zeaxanthin, is less responsive than wild type to BL, suggests that the BL response does not always correlate with bulk zeaxanthin. This finding is consistent with the presence of a small peak in the action spectrum at 360 nm, where the bulk zeaxanthin in the *trans* form does not absorb (Eisinger et al., 2000). However, it is possible that a small subset of the total zeaxanthin population is in the *cis* form and that this represents the physiologically active zeaxanthin BL photoreceptors. In fact, bulk zeaxanthin might actually inhibit the BL response, as occurs in the *aba1* mutant, as a result of shielding effects. This overall interpretation is supported by studies with the zeaxanthin-deficient mutant, *npq1*.

Frechilla et al. (1999) initially reported that stomata of *npq1* failed to open in response to BL, although it showed normal red light-induced opening. Subsequently, both Eckert and Kaldenhoff (2000) and Kinoshita et al. (2001) reported that under their conditions, the *npq1* mutant had normal BL-induced stomatal opening. In the present study, we were able to confirm the results of Frechilla et al. (1999) that the stomata of *npq1* do not respond to BL. As will be discussed later, the source of the discrepancy in the *npq1* results has now been resolved in a way that leaves the zeaxanthin hypothesis intact. In the meantime, however, Kinoshita et al. (2001) also demonstrated that a *phot1/phot2* double mutant lacked a BL response, leading them to conclude that phototropins, rather than zeaxanthin, serve as the BL receptors of guard cells. Thus, there are now two viable candidates for the BL photoreceptor of guard cells: zeaxanthin and the phototropins.

The first major finding of the current study is that stomata of both *npq1* and *phot1/phot2* open normally in response to UV-B. Because these mutants have normal UV-B-induced stomatal opening, we conclude that the UV-B response is mediated by a separate UV-B receptor. This conclusion is similar to that of a recent report on the regulation of chalcone synthase in *Arabidopsis* leaf tissue in which there are two regulatory pathways: a BL pathway involving *cry1* and a separate UV-B

pathway mediated by an unidentified UV-B receptor (Wade et al., 2001). The authors proposed a photoreceptor network for the regulation of chalcone synthase involving the UV-B receptor, cry1, phyA, and phyB (Wade et al., 2001). According to the model, phyB specifically antagonizes the UV-B receptor, while both phyA and phyB act synergistically with cry1.

The BL response of stomatal guard cells can be antagonized by GL (Frechilla et al., 2000; Talbott et al., 2002). The second major finding of the current report is that GL also antagonizes UV-B-induced opening. We also took advantage of the fact that both the *npq1* and *phot1/phot2* mutants opened their stomata in response to UV-B to test whether zeaxanthin or the phototropins could also serve as the GL photoreceptor. Although stomata of both mutants opened normally in response to UV-B, neither showed the normal GL antagonism of the UV-B response. Thus, we were able to show that both zeaxanthin and the phototropins are both required for GL inhibition of stomatal opening and that the UV-B receptor is separate from the GL photoreceptor.

As to the identity of the GL photoreceptor, once again the data do not allow us to choose between zeaxanthin and the phototropins because neither of the mutants respond to GL. Zeiger and colleagues (2000) have proposed a model for zeaxanthin analogous to the phytochrome Pr/Pfr photoreversible reaction. In vitro spectroscopic evidence that BL stimulation and GL inhibition are mediated by a reversible *cis-trans* isomerization of a pigment in chloroplast membrane has been obtained (Zeiger et al., 2000). When isolated thylakoids of *A. thaliana* were irradiated with alternating blue and green pulses, the difference spectrum exhibited a photoreversible bleaching in the blue region and an increase in absorption in the green and UV-A regions. Thylakoids from the zeaxanthin-deficient mutant *npq1* lacked the response, and the response could be reconstituted by adding exogenous zeaxanthin to the reaction, but not violaxanthin (Zeiger et al., 2000).

There is no known blue-green photoreversible reactions in phototropins 1 and 2 comparable to the *cis-trans* isomerization reaction described for zeaxanthin. Moreover, GL does not antagonize BL during phototropism as it does during stomatal opening (L. Taiz and W. R. Eisinger, unpublished data). Assuming that phot1 and phot2 are the photoreceptors for phototropism, the absence of GL antagonism in phototropism suggests very different photochemical reactions in the two processes. On the other hand, if zeaxanthin is the blue-green photoreversible photoreceptor, how does one explain the lack of GL antagonism in the *phot1/phot2* double mutant? If it is assumed that zeaxanthin is restricted to the chloroplast and phototropins are located in the cytosol, physical interaction between the two would seem to be ruled out. Alternatively, a signaling intermediate emanating from the chloroplast might interact either positively or negatively with phototropins, and both partners might be required for the blue-green reversible response. Because GL also antagonizes UV-B, this hypothetical interaction between the chloroplast signal and the phototropins must act downstream of the UV-B receptor, which would also explain why the effects of BL and UV-B are not additive, despite the existence of separate photoreceptors.

As noted earlier, the role of zeaxanthin in blue-light-induced stomatal opening has been challenged by two different laboratories. Both Eckert and Kaldenhoff (2000) and Kinoshita et al. (2001) observed normal stomatal opening in *npq1* in response to blue light. Their results conflict with the results presented here and with the results of Frechilla et al. (1999). More recently, it has been shown that the BL response, measurable under certain conditions, in the *npq1* mutant can be reversed by far-red light, suggesting the participation of phytochrome rather than the BL photoreceptor (E. Zeiger, UCLA, personal communication). Phytochrome is also known to be involved in stomatal opening in the orchid *Paphiopedilum*, whose guard cells lack developed chloroplasts (Talbott et al., 2002). However, far-red reversal is not observed in wild-type *Arabidopsis*, suggesting that the phytochrome mechanism of BL-induced opening is normally suppressed by the chloroplast-derived zeaxanthin pathway.

Interestingly, green light causes the opening of *Paphiopedilum* stomata, and green light-induced opening can be reversed by far-red light (Talbott et al., 2002). Thus, when the phytochrome pathway is activated (that is, when the zeaxanthin pathway is weak) the green-light response is mediated by phytochrome and is thus far-red reversible.

Could phytochrome be acting as the UV-B receptor? There are currently two arguments against this hypothesis: first, the UV-B stomatal opening pathway is active in the wild type under conditions when the phytochrome pathway appears to be inactive; second, green light acts as an inhibitor of stomatal opening in the UV-B pathway, whereas green light causes stomatal opening in the phytochrome pathway. In other words, green light has opposite effects on stomatal opening in the two pathways. It may be relevant in this regard that in the photoreceptor network proposed for chalcone synthase, PhyB acted as an inhibitor of the UV-B receptor (Wade et al., 2001).

Figure 11 shows a working model for the photoreceptor network proposed to regulate stomatal opening. A subset of zeaxanthin molecules, perhaps located on the chloroplast inner membrane, can undergo a photoreversible *trans-cis* isomerization in response to blue and green/UV-A light. In response to blue light, a positive signaling intermediate is transmitted to the cytosol where it interacts with phot1 and phot2. In turn, phot1 and phot2 act as the primary blue/UV-A photoreceptors, which bring about the activation of the plasma membrane H^+-ATPase involved in stomatal opening. Green light and UV-A drive the physiologically active zeaxanthin to the inactive *trans* state, which results in a negative signal in the cytosol, which blocks the action of phot1 and phot2. Because phot1 and phot2 must interact with the positive zeaxanthin signal, any mutation that blocks zeaxanthin synthesis would also block the action of phot1 and phot2. However, the same signal that activates phot1 and phot2 also appears to repress the action of phytochrome. Thus, when the zeaxanthin-signaling intermediate is blocked, as in the case of *npq1,* blue-light-induced opening can be antagonized by far-red light. Opening in the stomata of *Paphiopedalum*. which lack chloroplasts, can be stimulated by red light and green light as well, and in both cases the effect can be prevented by far-red light (Talbott et al., 2002).

Stomatal opening can also be regulated by a separate UV-B receptor. The UV-B receptor can activate the PM-ATPase independently of the positive zeaxanthin signal and of phot1 and phot2. This explains why both *npq1* and the *phot1/phot2* double mutants exhibit normal responses to UV-B. However, the UV-B response can be antagonized by both green light and UV-A. The GL response is absent in the *npq1* and *phot1/phot2* double mutants. This suggests that the negative zeaxanthin-signaling intermediate and the two phototropins act in series to inhibit the UV-B receptor.

Finally, it seems likely that such complex networks of pho-

toreceptors will turn out to be the rule rather than the exception in plant photomorphogensis. The control of flowering is another example of photoreceptor networks involving phytochrome and cryptochrome genes. The era of simple linear photoreceptor models would seem to be over.

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