PHYTOCHROME KINASE SUBSTRATE1 Regulates Root Phototropism and Gravitropism[1[C][W][OA]]

Hernán E. Boccalandro[2,3], Silvia N. De Simone[2], Ariane Bergmann-Honsberger, Isabelle Schepens, Christian Fankhauser, and Jorge J. Casal*

IFEVA, Facultad de Agronomía, Universidad de Buenos Aires and Consejo Nacional de Investigaciones Científicas y Técnicas, 1417-Buenos Aires, Argentina (H.E.B., S.N.D.S., J.J.C.); Center for Integrative Genomics, University of Lausanne, CH–1015 Lausanne, Switzerland (I.S., C.F.); and Department of Molecular Biology, University of Geneva, CH–1211 Geneva 4, Switzerland (A.B.-H.)

Light promotes the expression of *PHYTOCHROME KINASE SUBSTRATE1* (*PKS1*) in the root of *Arabidopsis thaliana*, but the function of PKS1 in this organ is unknown. Unilateral blue light induced a negative root phototropic response mediated by phototropin 1 in wild-type seedlings. This response was absent in *pks1* mutants. In the wild type, unilateral blue light enhanced *PKS1* expression in the subapical region of the root several hours before bending was detectable. The negative phototropism and the enhanced *PKS1* expression in response to blue light required phytochrome A (phyA). In addition, the *pks1* mutation enhanced the root gravitropic response when vertically oriented seedlings were placed horizontally. The negative regulation of gravitropism by PKS1 occurred even in dark-grown seedlings and did not require phyA. Blue light also failed to induce negative phototropism in *pks1* under reduced gravitational stimulation, indicating that the effect of *pks1* on phototropism is not simply the consequence of the counteracting effect of enhanced gravitropism. We propose a model where the background level of PKS1 reduces gravitropism. After a phyA-dependent increase in its expression, PKS1 positively affects root phototropism and both effects contribute to negative curvature in response to unilateral blue light.

Root tissues may be exposed to light due to light penetration into the upper layers of the soil (Mandoli et al., 1990) and tissue piping effects (Mandoli and Briggs, 1984). Whereas shoots bend toward the direction of incoming blue light, improving the chances of light-harvesting organs to collect light for photosynthesis, roots bend away from the direction of incoming blue light stimuli, avoiding the stressful conditions of the upper layers of the soil (Esmon et al., 2005). Phytochromes (Somers and Quail, 1995) and phototropins (Sakamoto and Briggs, 2002) are expressed in root as well as shoot tissues. Phototropin 1 (phot1) and phot2 are blue-light photoreceptors that play a major role in perception of the light gradient that initiates both shoot and root phototropism (Liscum and Briggs, 1995; Briggs and Christie, 2002). Roots of the *phot1* mutant are less efficient in exploring the soil because they show more frequent random turns than the wild type and therefore require more growth in length to achieve the same depth (Galen et al., 2006). An apparent consequence of the deficient growth pattern of *phot1* roots is the impaired plant biomass gain in dry soils, suggesting that genetic engineering of root negative phototropism could enhance productivity in arid environments (Galen et al., 2006).

Phytochromes A to E (phyA–phyE) are red-light and far-red-light photoreceptors that secondarily also absorb blue light. In higher plants, unilateral red or far-red light does not initiate phototropic responses in the shoot; however, red light induces weak positive phototropism in the root of Arabidopsis (*Arabidopsis thaliana*; Ruppel et al., 2001; Kiss et al., 2003b). In addition, phytochromes can modulate the ability of the hypocotyl to respond to the phototropic stimulus perceived by phototropins (Liscum and Briggs, 1996; Parks et al., 1996; Janoudi et al., 1997; Stowe-Evans et al., 2001). The root of *phyA* and *phyA phyB* mutants of Arabidopsis shows reduced response to unilateral blue light, a response that is unaffected by the *phyB* mutation (Kiss et al., 2003a).

PHYTOCHROME KINASE SUBSTRATE1 (PKS1) is a plasma membrane-associated protein (Lariguet et al., 2006) that physically interacts with and is phosphor-

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[2] These authors contributed equally to the article.

[3] Present address: Cátedra de Química Orgánica y Biológica, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Almirante Brown 500, 5505 Chacras de Coria, Mendoza, Argentina.

* Corresponding author; e-mail casal@ifeva.edu.ar.

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ylated by phytochromes in vitro (Fankhauser et al., 1999). PKS1 and its closest homolog, PKS2, are components of a complex network that modulates the very low-fluence-response branch of phyA signaling (Lariguet et al., 2003). PKS1 also binds phot1 and NONPHOTOTROPIC HYPOCOTYL3 (NPH3; Motchoulski and Liscum, 1999; Lariguet et al., 2006) and mutants deficient in PKS1, PKS2, and/or PKS4 (another member of the PKS1–PKS4 family in Arabidopsis) show severely reduced hypocotyl phototropism under low fluences of unilateral blue light (Lariguet et al., 2006). Blue light perceived by phyA induces expression of PKS1 in hypocotyls of Arabidopsis seedlings (Lariguet et al., 2006). Therefore, enhancement of the hypocotyl phototropic response by phyA (Parks et al., 1996; Janoudi et al., 1997) could result, at least in part, from the enhanced level of expression of PKS1 triggered by this photoreceptor. In the root, red light also promotes expression of PKS1, NPH3, and RPT2 (Molas et al., 2006), which are key players in tropic responses (Motchoulski and Liscum, 1999; Sakai et al., 2000).

The root shows a strong positive gravitropic response that orients growth toward deeper soil strata (Chen et al., 1999), where blue light no longer provides a signal (Mandoli et al., 1990). Light and gravitropic responses share some players downstream of the events related to signal perception and exhibit complex mutual interactions in the control of organ orientation (Correll and Kiss, 2002). Phototropic responses often involve deviation of the growth direction from the gravity vector and generate a gravitational stimulus that partially counteracts phototropism. Consequently, mutants with deficient gravitropic response show apparently enhanced root phototropism (Okada and Shimura, 1994; Vitha et al., 2000). In turn, light modulates the gravitropic response, but the effect can be positive or negative, depending on the species and organ (for review, see Correll and Kiss, 2002). In maize (*Zea mays*) roots, for instance, red light stimulates gravitropism (Feldman and Briggs, 1987). However, light perceived by phyA and phyB reduces the gravitropic response of the hypocotyls in Arabidopsis (Liscum and Hangarter, 1993; Poppe et al., 1996). Roots from *phyA phyB* or *phyB* mutants have reduced gravitropic response compared with the wild type (Correll and Kiss, 2005).

Although red and far-red light induce expression of *PKS1* in the root, no function of PKS1 in the root has been identified (Lariguet et al., 2003). PKS1 physically interacts with phot1 and NPH3 and regulates shoot phototropism (Lariguet et al., 2006), but root and shoot show different patterns of tropic responses. This scenario prompted us to investigate whether PKS1 is important for phot1-mediated root phototropism and for gravitropism.

RESULTS

Negative Root Phototropism Requires PKS1

To investigate whether PKS family members play a role in negative root phototropism, we provided unilateral blue light to etiolated vertically oriented seedlings of different single and double *pks* mutants. The two *pks1* mutant alleles used here presented no root curvature in response to 24 h of 1 μmol m^{-2} s^{-1} unilateral blue light (Fig. 1, A and B). *pks2* and *pks4* mutants showed wild-type root phototropism and the *pks1 pks2* and *pks1 pks4* double mutants behaved as the *pks1* single mutant (Fig. 1A). Transgenic lines overexpressing *PKS1*, *PKS2*, or *PKS4* also showed wild-type curvature in response to unilateral blue light (Fig. 1A). In wild-type seedlings, the degree of root curvature increased significantly with the fluence rate of blue light in the whole range tested here (0.003–10 μmol m^{-2} s^{-1}). However, neither the *phot1* mutant nor the *pks1* mutant showed detectable root phototropic response (Fig. 1C). Unilateral blue-light treatments (10 μmol m^{-2} s^{-1}) that failed to induce root phototropism in *pks1* and *phot1* mutants still caused significant positive phototropism of the hypocotyl (Fig. 1C, inset). As expected (Ruppel et al., 2001; Kiss et al., 2003b), unilateral red light (5 μmol m^{-2} s^{-1}) caused a weak positive phototropic response of the root. The *pks1* mutant failed to show this response (curvature, degrees, mean $\pm$ SE; *pks1* = 1 $\pm$ 1 against red light; wild type = 2 $\pm$ 1 toward red light).

Negative Root Phototropism and Blue-Light Induction of *PKS1* Expression Require phyA

The *phyA* mutant showed significantly reduced root curvature in response to unilateral blue-light irradiation (Kiss et al., 2003a), whereas *cryptochrome1* (*cry1*) and *cry2* mutants presented normal root phototropism (Fig. 2A). *PKS1* expression in the elongation zone of the root is promoted by white, red, or far-red light (Lariguet et al., 2003). To investigate whether the light stimulus that induces the negative phototropic response of the root also promotes activity of the *PKS1* promoter, seedlings bearing a *GUS* reporter transgene fused to the *PKS1* promoter were exposed to unilateral blue light. This light treatment strongly enhanced *PKS1* promoter activity in the root (Fig. 2B). The *PKS1-GUS* construction was introduced in the *phyA*, *cry1*, and *phot1* mutants by crosses. The absence of GUS staining in the *phyA* mutant and the normal staining observed in the *cry1* and *phot1* mutants indicates that the blue-light treatment inducing expression of the *PKS1-GUS* transgene was perceived largely by phyA (Fig. 2B).

Blue-Light Induction of *PKS1* Expression Anticipates Negative Root Phototropism

To investigate the kinetics of root curvature, 2-d-old, vertically oriented etiolated seedlings of the wild type and the *pks1* mutant were exposed to unilateral blue light (1 μmol m^{-2} s^{-1}). Root curvature was detected in wild-type seedlings 10 h later and reached a maximum after 20 h of treatment (Fig. 3A). The *pks1* mutant failed to respond. The effect of unilateral blue light on GUS driven by the *PKS1* promoter was already noted after

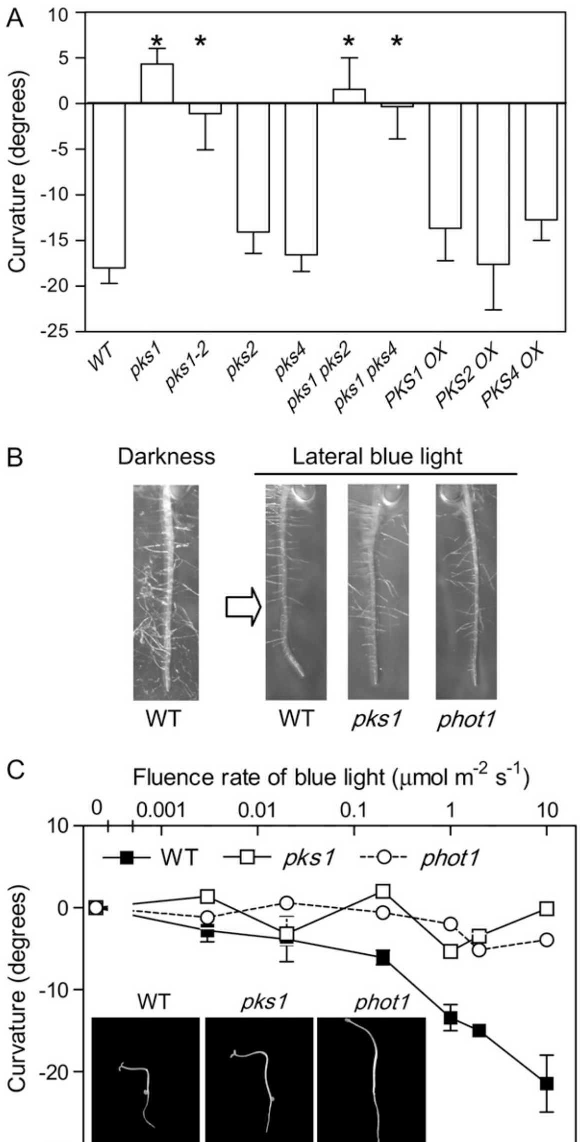

Figure 1. Root phototropic response to unilateral blue light requires PKS1. A, Reduced root phototropism in the *pks1*, *pks1 pks2*, and *pks1 pks4* mutants and normal responses in the *pks2* and *pks4* mutants and *PKS1*, *PKS2*, and *PKS4* overexpressors. B, Roots of representative seedlings. C, Response to fluence rate. Seedlings were grown in full darkness for 2 d and exposed to unilateral blue light for 24 h (1 μmol m^{-2} s^{-1} [A and B] or the indicated fluence rate in C) before measurements. Dark controls were measured simultaneously. Data are means and SE of 20 replicate boxes.

2 h of treatment (i.e. well before any phototropic curvature was detectable [Fig. 3B]). The *PKS1* response anticipated by at least 5 h the phototropic response.

The strongest GUS staining driven by the *PKS1* promoter was observed in the subapical zone of the root (Fig. 3B). Labeling the place that the root tip had reached at the time when the light treatment started revealed that this point coincided with the place where the curvature occurred; therefore, the initial expression of *PKS1* could act as a marker for the place of subsequent bending. GUS staining gradually extended to the rest of the elongation zone (4–7 h; Fig. 3B). After 24 h of blue-light treatment, staining extended to the curvature zone (Fig. 3B), but GUS stability might contribute to staining of these distant cells as they move away from the subapical region.

PKS1 Negatively Regulates Root Gravitropism

Phototropic and gravitropic responses share signaling components involved in the generation of the extension growth of the shoot and root axes (Correll and Kiss, 2002). To investigate whether, in addition to its effect on the negative phototropic response, PKS1 also affects root gravitropism, dark-grown seedlings of the wild type, the *pks1* mutant, and a *PKS1* overexpressor were exposed to either 1 h of red light followed by 11 h of darkness or to 12 h of red light, whereas dark controls remained without red-light treatment. Red light was provided from both sides to enhance *PKS1* expression (Supplemental Fig. S1A), while avoiding induction of the phototropic response. In the wild type, the angle between the root and the gravity vector (randomization of root growth direction) was not affected by 1 h of red light compared to dark controls, but 12 h of red light significantly randomized root position and therefore increased the average deviation compared to the gravity vector (Fig. 4A). The *pks1* mutant showed normal root angle in darkness or after 1 h of red light, but it failed to reduce the gravitropic orientation in response to 12 h of bilateral red light. The *PKS1* overexpressor showed constitutive enhanced deviation in darkness without a significant response to red light. In additional experiments, a similar pattern was observed for a second, independent *PKS1* overexpressor line (deviation from gravity vector in darkness, degrees, mean ± SE; *pks1* = 7 ± 1; wild type = 9 ± 1; *PKS OX* = 21 ± 3; PKS OX′ = 21 ± 3). Noteworthy is the fact that the *phot1* mutant behaved as the *pks1* mutant (i.e. it retained strong vertical orientation even after 12 h of red light).

In a second experimental setting, dark-grown seedlings were shifted from the vertical to the horizontal position after 0, 1, or 12 h of bilateral red-light (Fig. 4B) or blue-light (Fig. 4C) treatment. A negative correlation between PKS1 levels and gravitropic response occurred in all the latter conditions, including darkness. In dark-grown seedlings, there is detectable *PKS1* expression in the root, but resolution of the system does not allow us to conclude whether *PKS1* expression is enhanced by the gravitropic stimulus. The *phyA* mutation did not enhance the gravitropic response in our conditions (Fig. 4C). Exposure to 1 h of red light increased *PKS1* expression in the root (Supplemental Fig. S1B) and also enhanced the gravitropic response in all genotypes (Fig. 4B). Compared to 1 h of red light, exposure to 12 h of red light reduced the gravitropic response in the wild type, particularly in the *PKS1 OX* line, but not in the *pks1* and *phot1* mutants (Fig. 4B). In

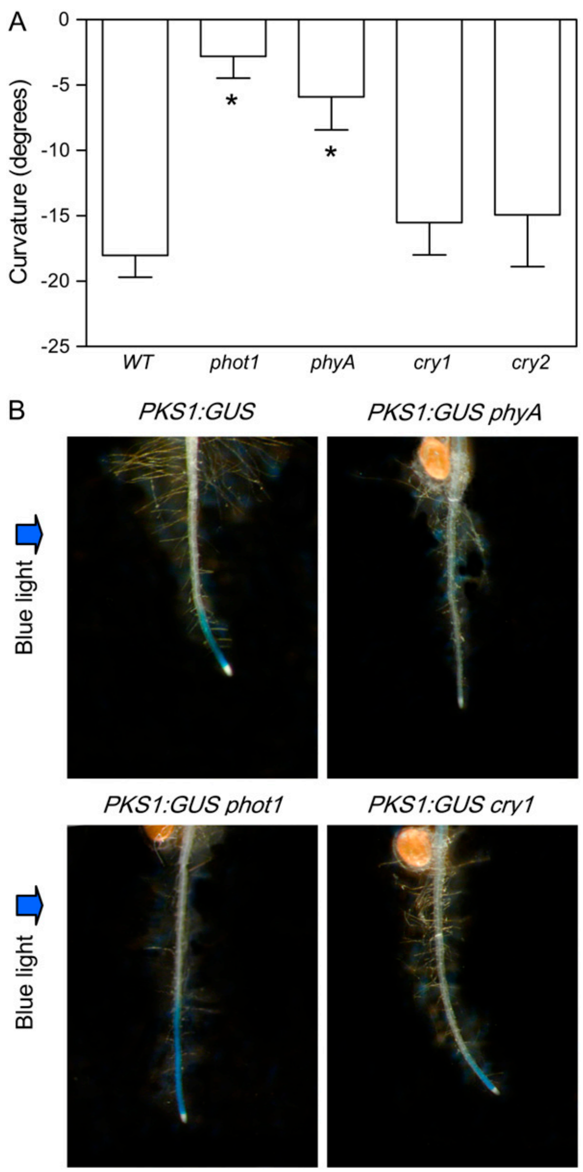

Figure 2. Negative phototropism and promotion of *PKS1* promoter activity require phyA. A, Reduced phototropism in *phyA* and *phot1* mutants. Data are means and SE of 10 replicate boxes. B, Reduced induction of GUS driven by the *PKS1* promoter in the root of *phyA* mutant seedlings. Seedlings were grown in full darkness for 2 d, transferred to unilateral blue light (1 μmol m^{-2} s^{-1}), and measured or stained 24 h later.

accordance with the experiments described in the above paragraph, the *phot1* mutant behaved as the *pks1* mutant. In darkness, the effects of *phot1* and *pks1* were additive (Fig. 4C).

Negative Root Phototropism Requires PKS1 Even under Reduced Gravitational Stimulus

Seedlings of the *phosphoglucomutase* (*pgm*; Caspar and Pickard, 1989) and *altered response to gravity* (*arg*; Sedbrook et al., 1999) mutants, which have deficient gravitropic responses, were exposed to unilateral blue light. The phototropic bending induced by unilateral blue light was significantly higher in *pgm* (27.9 ± 1.0 degrees) and *arg* (25.7 ± 1.5 degrees) than in the wild type (16.6 ± 0.6 degrees). This and previous observations (Okada and Shimura, 1994; Vitha et al., 2000) suggest that the reduced phototropic response of *pks1* might be caused by its enhanced gravitropism. To investigate this possibility, seedlings were incubated in a clinostat to abolish gravitational stimulation. Seedlings were rotated on an axis perpendicular to the gravity vector. In dark controls, the direction of root growth did not deviate significantly from random (Fig. 5). In wild-type seedlings exposed to 12 h of unilateral blue light, the root deviated significantly from random, adopting a position close to parallel to the irradiation axis (90 degrees in Fig. 5). When exposed to unilateral blue light, the root of the *pks1* mutant retained a close-to-random position (Fig. 5), indicating that the phototropic response was severely reduced in *pks1* even under reduced gravitational stimulus.

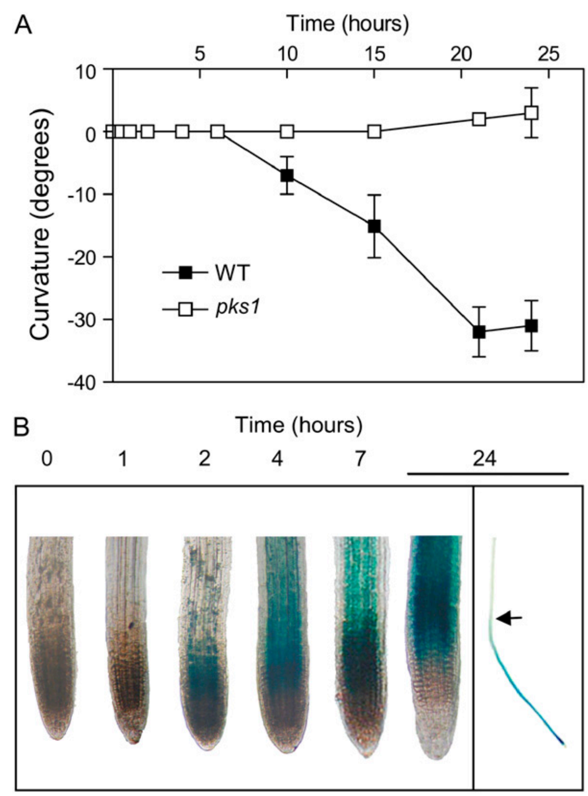

Figure 3. Changes in *PKS1* promoter activity anticipate PKS1-mediated effects on negative phototropism in roots. A, Time course of root bending after the beginning of unilateral blue light. Data are means and SE of nine replicate boxes. B, Time course of GUS staining driven by the *PKS1* promoter under unilateral blue light. In B, magnification is 100× (left box) and 7× (right box). The arrow indicates the position of the root tip at the beginning of the light treatment (this position was labeled in some boxes under dim green light). Seedlings were grow in full darkness for 2 d, transferred to unilateral blue light (1 μmol m^{-2} s^{-1}), and measured or stained at the indicated time point.

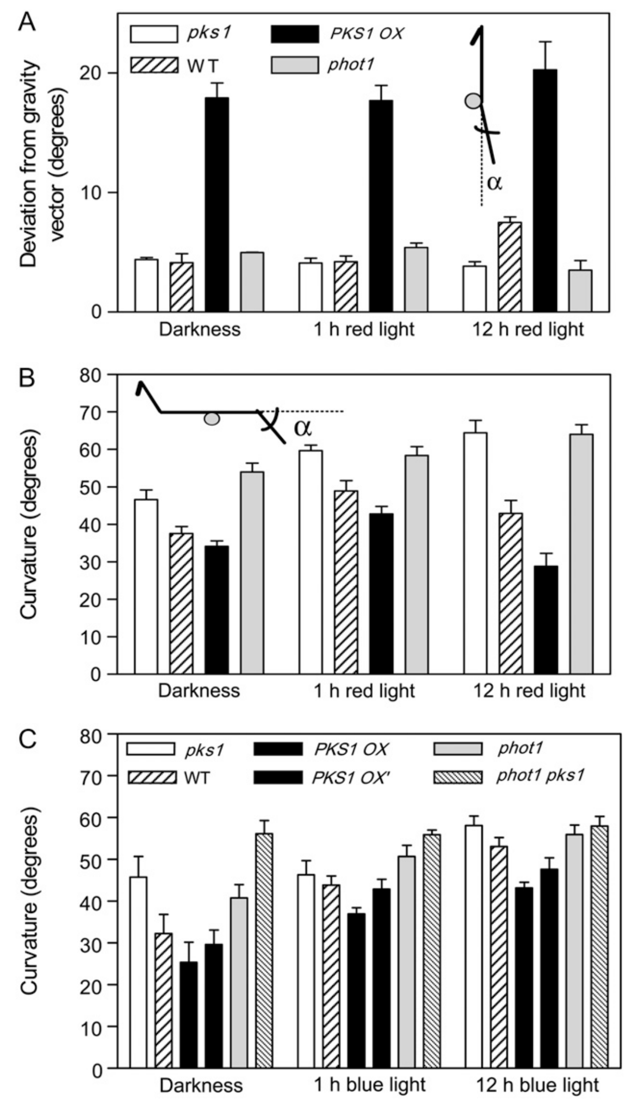

Figure 4. PKS1 negatively regulates root gravitropism. A, Root deviation from gravity in vertically grown seedlings exposed to 0, 1, or 12 h of bilateral red light (5 μmol m^{-2} s^{-1}). Seedlings were grown vertically for 2 d and exposed to 1 h of bilateral red light followed by 23 h of darkness, 12 h of bilateral red light followed by 12 h of darkness, or left as dark controls. B, Deviation from the horizontal plane (gravitropic response) in seedlings transferred from the vertical to the horizontal position. Seedlings grown vertically for 2 d were transferred to the horizontal position and the change in root growth angle was measured 24 h later. Some seedlings were exposed to 1 h or 12 h of bilateral red light (5 μmol m^{-2} s^{-1}) immediately prior to gravitropic stimulation. C, Experimental setting, as in B, but using bilateral blue light (1 μmol m^{-2} s^{-1}). Data are means and SE of at least five replicate boxes.

DISCUSSION

PKS1, originally discovered by its ability to interact with and become phosphorylated by phytochromes in vitro (Fankhauser et al., 1999), was later shown to interact also with phot1 and NPH3 (Lariguet et al., 2006). PKS1 and PKS2 regulate phytochrome-mediated photomorphogenesis (Lariguet et al., 2003) and hypocotyl phototropism (Lariguet et al., 2006). However, despite the observation that *PKS1* is expressed in the roots in response to pulses of red or far-red light, no root phenotype had become obvious (Lariguet et al., 2003). The results presented here indicate that PKS1 positively regulates negative root phototropism induced by unilateral blue light and negatively regulates root gravitropism. These findings place the PKS family as central players in the control of organ orientation.

Seedlings of *pks1* mutants exposed to 12 h of continuous blue light of up to 10 μmol m^{-2} s^{-1} from one side failed to show root curvature (Fig. 1). *pks2* and *pks4* mutants showed normal negative phototropism. The latter is different from the scenario observed for shoot phototropism, where PKS2 and PKS4 play a similar and partially redundant role, with PKS1 promoting hypocotyl phototropism (Lariguet et al., 2006), but it is consistent with the observation that at least *PKS2* and *PKS4* are expressed exclusively in aerial tissues (Lariguet et al., 2003; I. Schepens and C. Fankhauser, unpublished data). Unilateral blue light induces *PKS1* expression in the root several hours before any root curvature response becomes detectable (Fig. 3). The place of early *PKS1* expression coincides with the position where root curvature is observed later, indicating that PKS1 could be an early location marker. No radial gradient of *PKS1* expression in response to unilateral blue light was apparent. This suggests that PKS1 is not acting directly in the main signaling stream downstream of phot1, which shows an apparent gradient of activation in response to unilateral blue light (Salomon et al., 1997), but rather as a regulator of these signaling events downstream of phot1. After introducing the *PKS1-GUS* transgene in the *phyA*, *phot1*, and *cry1* mutant backgrounds, we

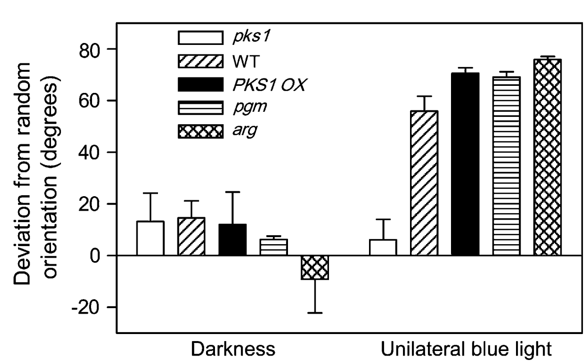

Figure 5. Negative phototropism of the roots requires PKS1 even under reduced gravity. Clear plastic boxes containing chilled seeds were transferred to the clinostat (two revolutions/min) at 22°C and exposed for 12 h to white light to induce germination. Then, boxes were wrapped in black plastic (full darkness) or in black plastic with a window to allow unilateral irradiation with blue light (1 μmol m^{-2} s^{-1}) normal to the axis of rotation of the clinostat. We measured the angle of the root to the gravitational axis. In dark-grown seedlings, the average angle tends to zero because divergent angles of deviation cancel each other. In the presence of unilateral blue light, the root grows parallel to the light axis. Data are means and SE of three (darkness) or six (blue light) replicate boxes.

conclude that phyA and not phot1 or cry1 mediates the response of *PKS1* expression to unilateral blue light (Fig. 2B) similarly to what has been observed in shoots (Lariguet et al., 2006). In agreement with previous reports (Kiss et al., 2003a), the negative phototropic response of the root was reduced in the *phyA* mutant (Fig. 2A). Taken together, these observations indicate that blue light perceived by phyA increases *PKS1* expression in the subapical region of the root, where bending is observed hours later, and this increase in *PKS1* expression is necessary for the normal phototropic response, providing a nice example of coordination between phyA and phot1 activity. Coordination between phytochrome and phototropin appears particularly important for photomovement responses as the fern *Adiantum capillus-veneris* and the filamentous green alga *Mougeotia scalaris* bear chimeras of these photoreceptors, which have evolved independently (Suetsugu et al., 2005).

In addition to its effects on root phototropism, PKS1 also regulated root gravitropism (Fig. 4). Analysis of randomization of root growth in vertically grown seedlings and quantification of the curvature of the root in seedlings shifted from the vertical to the horizontal position revealed negative correlation between *PKS1* levels (*pks1* mutant, wild type, *PKS1 OX*) and gravitropic response in dark-grown, bilateral red-light-treated and blue-light-treated seedlings. In gravity-stimulated seedlings, promotion of *PKS1* expression by red or blue light (Supplemental Fig. S1B; Fig. 2) is apparently not necessary for negative regulation of the gravitropic response by PKS1. First, red and blue light enhanced *PKS1* expression, but not the difference between the wild type and *pks1*, with the exception of 12 h of red light (Fig. 4, B and C). Second, promotion of *PKS1* expression by blue light requires phyA (Fig. 2B), but the *phyA* mutant showed no enhanced gravitropic response under blue light (Fig. 4C).The literature contains reports of positive as well as negative effects of light on gravitropism (for review, see Correll and Kiss, 2002). Here, we show that 12 h compared to 1 h of red light randomized root position in vertically grown seedlings (Fig. 4A) and reduced the curvature in gravity-stimulated seedlings (Fig. 4B) in the wild type but not in the *pks1* mutant. Therefore, positive and negative effects of light on gravitropism can be separated temporarily and genetically in the same system.

Noteworthy is the fact that the *phot1* mutant showed enhanced gravitropism not only in seedlings exposed to bilateral blue light, but also in seedlings grown in darkness or exposed to bilateral red light (i.e. in the absence of blue-light activation). This phenotype is manifested as reduced root deviation from the gravity vector in vertically grown seedlings (Fig. 4A) and increased curvature of the root when seedlings were placed horizontally (Fig. 4B). Clock (Devlin and Kay, 2000) and de-etiolation (Botto et al., 2003) phenotypes in the absence of blue light have also been reported for blue-light photoreceptor cryptochromes. PKS1 and phot1 interact physically (Lariguet et al., 2006) and both are

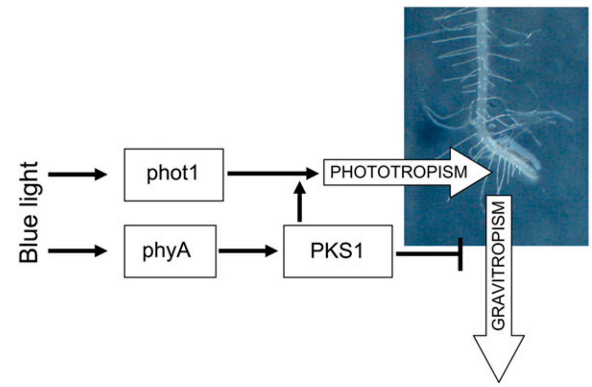

Figure 6. Working model of PKS1 function in root phototropism and gravitropism. Black arrows indicate positive regulatory processes and blunt-ended lines indicate negative regulatory processes. White arrows indicate phototropic and gravitropic forces acting in the direction of root growth. phyA-mediated induction of PKS1 is important for PKS1-mediated regulation of phototropism (as shown in Fig. 2), but not for the PKS1-mediated effect on gravitropism (as shown in Fig. 4). [See online article for color version of this figure.]

required to induce root phototropism (Fig. 1) and reduce root gravitropism (Fig. 4); however, not all of these activities may require light activation of phot1.

Phototropic responses involve bending either toward or against the light gradient with consequent deviation from the gravity vector. As a result, the actual degree of bending depends on the phototropic response and a counteracting gravitropic response (Okada and Shimura, 1994; Vitha et al., 2000). The positive effect of PKS1 on the phototropic response could therefore be due, at least in part, to its negative effect on gravitropism (Fig. 6). However, the *pks1* mutant failed to respond to blue light even under reduced gravitational stimulation (Fig. 5). This indicates that, in addition to the predicted indirect contribution of PKS1 to the phototropic response via its negative regulation of gravitropism, there is a more direct effect on phototropism itself (Fig. 6). The responses to gravity and unilateral blue light share late steps connected to the generation of a gradient of auxin and the modification of the direction of the growth response (for review, see Correll and Kiss, 2002). The contrasting effects of PKS1 on root phototropism and gravitropism suggest that PKS1 regulates these processes upstream of their convergence.

MATERIALS AND METHODS

Plant Material

The *pks1-1* (Lariguet et al., 2003), *pks1-2*, *pks2-1* (Lariguet et al., 2003), *pks4-1* (Lariguet et al., 2006), *phot1-5* (Huala et al., 1997), *cry1-304*, *cry2-1* (Guo et al., 1998), and *phyA-211* (Nagatani et al., 1993) mutants and the *PKS1 OX*, *PKS2 OX*, and *PKS1*::*GUS* transgenic lines (Lariguet et al., 2003) used here are in the Arabidopsis (*Arabidopsis thaliana*) Columbia background. *PKS4*-overexpressing plants were obtained by transforming Columbia-0 seedlings with construct pIS007, which codes for the PKS4 cDNA driven by the cauliflower mosaic virus 35S promoter. *PKS4* coding sequence flanked with *Bam*HI sites was amplified by PCR (IS01 5′-gga tcc atg gcg caa act act gtc ac-3′ and IS02 5′-gga tcc tgg tat cca tca ttg cct tg-3′), and the *Bam*HI-digested product was ligated into *Bam*HI-digested pCGN18 to yield pIS007. All PCR-generated constructs were verified by sequencing. Two single insertion lines expressing elevated levels of *PKS4* mRNA (data not shown) were selected for further analysis (IS07-5 and IS07-11). We obtained the *pks1-2* insertion mutant from the GABI collection (line GABI 481C08; Rosso et al., 2003). T-DNA is inserted after the 53rd codon. No PKS1 was detected in *pks1-2* (Supplemental Fig. S2). The *PKS1*:*GUS* transgene was introduced in different mutant backgrounds by crossing. *PKS1*:*GUS phyA-211* was selected as tall seedlings under continuous far-red light (10 μmol m^{-2} s^{-1}) in the F_2 and F_3 generations. *PKS1*:*GUS cry1* was selected as tall under blue light (20 μmol m^{-2} s^{-1}) in the F_2 and F_3 generations. *PKS1*:*GUS phot1-5* was selected as nonbending seedlings under lateral blue-light irradiation (1 μmol m^{-2} s^{-1}) in the F_2 and F_3 generations. Screening for the presence of a homozygous *PKS1*:*GUS* transgene was performed by sowing seeds on petri dishes containing one-half-strength Murashige and Skoog medium and selecting 100% BASTA resistance and 100% GUS-stained seedlings under white light in the F_3 generation.

Light Treatments and Growth Conditions

Seeds were surface sterilized, sown on 0.8% agar-water in clear plastic boxes (42 × 35 mm^2 × 20 mm), incubated in the dark at 4°C for 3 d, and exposed to 1 h of red light to induce homogeneous germination. Then, boxes were placed with the agar oriented vertically and kept in the dark for 2 d at 22°C before light treatments. A combination of fluorescent tubes and a blue filter (Rosco; filter no. 83) provided blue light (λmax = 440 nm). Different blue-light fluence rates were obtained by interposing different numbers of neutral filters and measured with a LiCor radiometer. The spectral photon distribution of the blue-light field was measured with a spectroradiometer (Analytical Spectral Devices Field Spec Pro FR). Fluorescent tubes in combination with red, yellow, orange (Lee filters; nos. 106, 101, and 105, respectively) and neutral filters provided bilateral red light (λmax = 640 nm; 5 μmol m^{-2} s^{-1}).

Measurements of Curvature

Seedlings grew along the agar surface of the vertically oriented boxes. In phototropism experiments, the root of seedlings that grew toward the light source were given positive angles and the roots that grew away from the light were assigned negative angles. In gravitropism experiments, the angle between the gravitational vector and the root was assigned a positive value. Seedlings were photographed with a digital camera connected to a binocular loop. Images were used to measure the angle of root-growing direction against gravity vector using Image Tool Version 3 software from UTHSCSA. Each experiment was conducted at least on three independent occasions. Data from 10 seedlings were averaged per box (one replicate) and used for statistics (one-way ANOVA followed by Tukey's multiple comparison test or *t* test).

GUS Staining

GUS staining was conducted as described (Lariguet et al., 2003). Seedlings were observed with a binocular loop or an optical microscope and photographed with a digital camera. To better visualize and get an improved contrast between the seedling shape and the background, images were processed with a Photoshop program replacing the background of the original photograph by white or black color.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Expression of *PKS1* in root gravitropism experiments.

Supplemental Figure S2. Protein blot showing the failure of *pks1-1* and *pks1-2* mutants to accumulate PKS1.

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