Relocalization of the PIN1 Auxin Efflux Facilitator Plays a Role in Phototropic Responses[1][w]

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Recently, we reported that the basal localization of the PIN1 auxin efflux facilitator protein is disrupted in hypocotyls of Arabidopsis *mdr* (*pgp*) mutants grown in the dark or unidirectional light (Noh et al., 2003). Molecular genetic and physiological evidence indicates that PIN1 is required for transport of auxin from shoot to root apices (Okada et al., 1991; Friml and Palme, 2002), whereas immunohistochemical studies localize PIN1 to the lower ends of xylem-associated cells in both shoots and roots (Gälweiler et al., 1998). More recently, asymmetric PIN1 localization in root tips has been shown to involve dynamic cycling mediated by the ARF-GEF GNOM (Geldner et al., 2001, 2003). Surprisingly, although disruption of *MDR/PGP* genes results in decreased polar auxin transport (Noh et al., 2001; Geisler et al., 2003; Multani et al., 2003) and decreased free auxin content in lower shoot and root tissues (J.J. Blakeslee and A.S. Murphy, unpublished data), *mdr1 pgp1* mutant hypocotyls exhibit exaggerated tropic bending (Noh et al., 2003). According to the Cholodny-Went hypothesis (for summary, see Went, 1974), tropic bending is mediated by lateral redistribution of auxin near the site of tropic stimulus. Our report suggested that the enhanced tropic bending observed in *mdr/pgp* mutants resulted from decreased vertical auxin transport and a consequent increase of lateral auxin bias. However, it was not clear whether the perturbation of PIN1 localization in *mdr/pgp* mutants was due to disruption of an immediate interaction required for asymmetric localization of the PIN1 protein or from cumulative developmental defects resulting from altered auxin transport. The extent to which altered localized auxin levels might contribute to PIN1 delocalization in tissues where *AtMDR1* and *AtPGP1* are not expressed was also not determined. To determine whether PIN1 delocalization similar to that seen in hyper-phototropic *mdr/pgp* mutants also plays a role in the normal phototropic response, we investigated changes in PIN1 localization after initiation of the first positive phototropic curvature in wild-type (WT) seedlings responding to 450 nm of blue light.

When illuminated with directional blue light, Arabidopsis seedlings exhibit a phototropic bending response that involves two steps—an initial halt in vertical growth and subsequent initiation of bending (Parks et al., 2001). A signal transduction pathway initiated by phototropin perception of blue light (Briggs et al., 2001) and modulated by both Ca^{2+} (Harada et al., 2003; Stoelzle et al., 2003) and protein signaling components (Motchoulski and Liscum, 1999) has been shown to mediate the bending response. Although directional blue light is thought to be perceived in the upper portion of the hypocotyl (Parks et al., 2001), bending manifests in the mid-hypocotyl region and is thought to be regulated by auxin efflux through the laterally oriented PIN3 auxin efflux facilitator (Friml et al., 2002).

When WT seedlings were grown in the dark, where basal localization of PIN1 was observed (Fig. 1A), and subsequently exposed to unidirectional blue light (0.5 μmol m^{-2} s^{-1}, 450 nm, for 1.5 h), we observed phototropic bending of seedlings similar to that previously described (Briggs et al., 2001; Friml et al., 2002). After blue light stimulus, tissues were rapidly fixed, and both PIN1 and PIN3 proteins were localized by immunofluorescence microscopy. Although no changes in PIN3 localization could be detected (data not shown), we observed PIN1 delocalization (Fig. 1B) in the mid-hypocotyl region where phototropic bending occurs and has been shown previously to accumulate auxin as part of that response (Friml et al., 2002). A gradient in PIN1 delocalization was observed, with a disruption of basal localization of PIN1 on the side of the seedling distal to the light source (Fig. 1B). To confirm that this was a blue light-dependent phenomenon, we subjected *phot1* mutants to the same blue light treatment. Although it was more difficult to visualize PIN1 by immunofluorescence in Col-0 hypocotyls, patterns of PIN1 localization in dark-grown (Fig. 1C) and blue light-treated Col-0 hypocotyls resembled those observed in Ws (Fig. 1A). However, PIN1 localization in blue light-treated *phot1* mutant hypocotyls (Fig. 1D) resembled that seen in untreated WT Col-0 hypocotyls (Fig. 1C), demonstrating the strict blue light dependence of the observed changes in

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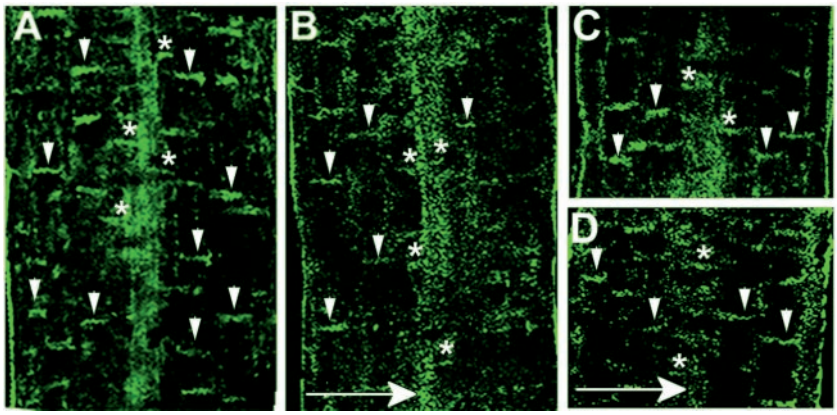

Figure 1. Delocalization of PIN1 in response to blue light treatment. Immunofluorescence confocal images of PIN1 localization in 4-d WT and *phot1* mutant seedlings exposed to unidirectional blue light. A, PIN1 is basally localized in dark-grown WT (Wassilewskija [Ws]) hypocotyls. B, Hypocotyl regions of 4-d Ws seedlings exposed to unidirectional blue light show decreased basal localization in cortical cells on the side distal to the light source. C, PIN1 basal localization in WT (Columbia-0 [Col-0]). D, PIN1 localization in blue light-treated *phot1* mutants remains undisrupted. Asterisks and arrowheads, Vascular and cortical localizations, respectively. Arrow, Direction of blue light.

PIN1 localization. These results provide evidence that disturbance of PIN1 basal asymmetry plays a role in the phototropic lateral redistribution of auxin.

Although the observed changes of PIN1 localization in the region of phototropic bending in blue light-treated seedlings could be anticipated from previous studies (Noh et al., 2003), an unexpected result was an observed randomization of PIN1 immediately below the region of phototropic curvature. In this region, PIN1 was delocalized in the vascular cells and exhibited a generalized loss of asymmetric distribution in cortical cells (Fig. 2A). Interestingly, a very similar delocalization of PIN1 in this region of the hypocotyl was also observed in *mdr1 pgp1* mutants (Noh et al., 2003; Fig. 2B), even in light-grown tissues where *MDR1* and *PGP1* are not expressed (Sidler et al., 1998; Noh et al., 2001). We hypothesized that the observed phenomenon might contribute to the initial halt of vertical growth observed in phototropic bending (Parks et al., 2001). Because we also have been unable to demonstrate direct MDR/PGP-PIN interactions in yeast two-hybrid and pull-down assays (data not shown), we hypothesized that the observed uniform dissociation might be initiated by alteration of auxin movement from the adjoining apical tissues.

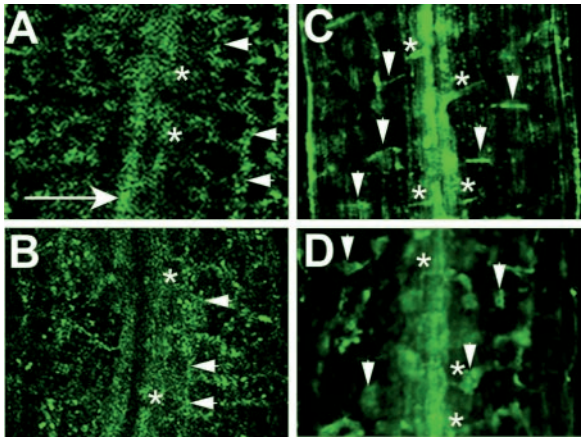

Figure 2. PIN1 localization in lower hypocotyls. Immunofluorescence confocal (A and B) and epifluorescence (C and D) images of PIN1 in lower hypocotyls. A, Basal PIN1 localization is disrupted after blue light treatment of 4-d dark-grown Ws hypocotyls. B, *mdr1 pgp1* mutants show a similar pattern of PIN1 delocalization. C, PIN1 localization in the lower hypocotyl of light-grown 5-d WT (Ws) seedlings. D, Disruption of basal PIN1 localization in Ws seedlings treated with auxin microdroplet applied to shoot tip. Asterisks and arrowheads, Vascular and cortical localizations, respectively. Arrow, Direction of blue light.

The obvious supposition is that a decrease of basipetal auxin movement to the mid-hypocotyl region disrupts PIN1 localization. Defects in basipetal auxin transport have been reported in *mdr/pgp* mutants (Noh et al., 2001; Geisler et al., 2003). The delocalization of PIN1 observed in photostimulated WT seedlings would be expected to lead to a similar, but temporary, decrease in vertical auxin transport. If this were true, then the delocalization of PIN1 observed in *mdr1 pgp1* mutant and photostimulated WT lower hypocotyls may result from decreased auxin levels in this region.

To test this hypothesis, we inhibited auxin transport using the auxin efflux inhibitors naphthylphthalamic acid (NPA) and triiodobenzoic acid (TIBA). To avoid direct interference of NPA and TIBA with protein-protein interactions in the mid-hypocotyl region, auxin transport from the shoot apex was inhibited by placing a 0.1-μL agarose droplet containing 10 μM NPA or 10 μM TIBA on the shoot apical tip. Preliminary experiments using radiolabeled NPA demonstrated that NPA applied to the shoot apex is restricted to the zone of application and does not move freely through seedling tissues in the time frame of this experiment. The efficacy of inhibitor application in reducing auxin flux was confirmed by auxin quantification, auxin transport assays, and DR5::GUS expression studies (data not shown). Unexpectedly, treatment with auxin transport inhibitors failed to cause delocalization of *PIN1* in WT tissues (data not shown). Furthermore, expression levels of *PIN1*, quantified by real-time PCR, were unchanged in NPA- or TIBA-treated shoot tissues (see Supplementary Table I, available in the online version of this article at http://www.plantphysiol.org).

As expected, free auxin levels in *mdr1* and *mdr1 pgp1* mutants are decreased in lower hypocotyls and slightly increased in upper hypocotyls compared with WT (J.J. Blakeslee and A.S. Murphy, unpublished data). Sufficient auxin accumulation to induce visible DR5::GUS activity also has been reported in photostimulated WT upper hypocotyls (Friml et al., 2002). Because of this, we reasoned that the generalized PIN1 delocalization seen in lower hypocotyls may result from increased auxin accumulations in the tissues immediately above. Increased auxin content in upper hypocotyls was simulated by placing a 0.1-μL microdroplet containing 1 μM indole-3-acetic acid on the apical tips of 5-d-old seedlings. Rates of transport were previously determined using [^{3}H]-indole-3-acetic acid as a tracer to select seedlings at a time point before the applied auxin pulse was evident in the mid-hypocotyl (Geisler et al., 2003). Increased auxin accumulation in the upper hypocotyls resulted in increased, not decreased, *PIN1* expression in shoot tissues (see Supplementary Table II). After auxin treatment, the lower hypocotyl region corresponding to the region immediately below the point of bending in blue light-treated seedlings (Fig. 2A) was examined. Compared with untreated controls (Fig. 2C), auxin-treated seedlings exhibited delocalization of PIN1 in the cortical tissues flanking the vascular bundle (Fig. 2D) similar to that observed in *mdr1 pgp1* mutants (Fig. 2B) and photostimulated WT seedlings.

DISCUSSION

The data presented here support the hypothesis that alterations of PIN1 localization contribute to the inhibition of vertical growth and the initiation of bending in auxin-mediated phototropic responses. The overall delocalization of PIN1 observed after blue light treatment appears to function as a mechanism to reduce basipetal auxin transport and sequester auxin at the expanding cells involved in phototropic bending. Because changes in PIN3 localization could not be visualized, it is unclear to what extent PIN3 relocalization contributes to the regulation of lateral auxin movement in tropic bending. Because delocalization of PIN1 was observed uniformly in cortical tissues of blue light-treated lower hypocotyls, where no accumulation of auxin is evident in the phototropic response (Friml et al., 2002), this effect in lower hypocotyls may be induced by another signal that precedes and modulates the effects of auxin pulses. It is interesting to note that a transitory increase in shoot auxin levels (Bhalerao et al., 2002; Casimiro et al., 2003) immediately precedes the disappearance of basal PIN1 localization in cortical cells of lower shoot tissues at 5 to 6 d (data not shown). This increase may also trigger other shoot developmental signals. Previous studies have suggested a role for a Ca^{2+}-modulated anion channel in the blue light response of Arabidopsis hypocotyls (Cho and Spalding, 1996; Lewis et al., 1997). A mobile signaling mechanism has also been proposed to function in the inhibition of shoot branching in both pea (*Pisum sativum*) and Arabidopsis (Beveridge et al., 2000; Booker et al., 2003; Sorefan et al., 2003). There is some evidence for involvement of MDR/PGP proteins in mediating such a response because *AtMDR1* was originally identified in screens utilizing the anion channel blocker NPPB (Noh et al., 2001).

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