ABSTRACT

DIS1 AND *DIS2* PLAY A ROLE IN TROPISMS IN *ARABIDOPSIS THALIANA*

by James Christopher Reboulet

To further understand the role of the cytoskeleton in gravitropism, we performed orientation and curvature studies on seedlings of *distorted1* (*dis1*) and *distorted2* (*dis2*) mutants of *Arabidopsis thaliana* which are defective in the ARP (actin related protein) 2/3 complex. Recently, ARP 2/3 has been studied for its effects on cell morphology in trichomes. DISTORTED mutants are phenotypically characterized by defects in trichome branching, vesicle localization, and F-actin organization. Because ARP 2/3 is expressed throughout all tissues, we hypothesized a presence in gravisensing cells of hypocotyls and roots. We found that *DIS1* enhanced gravitropism in roots of dark-grown seedlings, while *DIS2* attenuated gravitropism in roots grown in the light. We also found that both *DIS1* and *DIS2* attenuated phototropism in hypocotyls grown in the dark, while in roots, *DIS1* attenuated phototropism. This suggests a role for ARP 2/3 in cytoskeletal organization and these tropistic signaling pathways.

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INTRODUCTION

Gravity is a constant, ubiquitous force that has influenced plant development throughout its evolutionary history. The change in the direction of plant growth in response to gravity is termed gravitropism. In general, roots tend to exhibit positive gravitropism (i.e. they grow toward the gravity vector), and shoots exhibit negative gravitropism by growing away from the gravity vector. An alternative way of viewing gravitropism is to define it in terms of a gravity set-point angle (GSPA); Kiss, 2000. According to this view, promoted by Digby and Fern (1995), plant organs attempt to maintain constant alignment with their predefined GSPA, that is, the angle between the plant organ in question and the gravity vector. GSPA is generally species- and organ-specific and requires that the organ be able to detect a deviation from its GSPA and effect a counter response in the opposite direction to realign with its GSPA (Blancaflor and Masson, 2003). Although gravitropism has been well studied, the specific cellular mechanisms by which plants sense the direction of gravity and respond by altering their growth remain unresolved.

Gravitropism can be divided into the following three processes: perception, signal-transduction, and response (Kiss, 2000). Gravity perception, or the plant's sensing of a change in the gravity vector, occurs in the central columella cells within the root cap, and in stems, occurs in the endodermal cells, surrounding the vascular cylinder. The cells in the zone of elongation of roots are hypothesized to grow at differential rates, leading to the bending phenomenon, which constitutes the response phase. The mechanisms of the transduction phase, where the gravity stimulus is transmitted into biochemical signals which are carried to the putative responding cells are largely unknown.

Two models of gravity perception in plants, which have recently been the most pervasive, are the starch-statolith hypothesis and the protoplast-pressure hypothesis. The starch-statolith hypothesis proposes that starch-filled amyloplasts (statoliths) within gravity-perceiving cells (statocytes) fall toward the bottom of the statocytes. Displacement of the statoliths causes a signal to be transduced, which ultimately results in a differential response to

gravity. On the contrary, the protoplast-pressure hypothesis suggests that the entire mass of the cytoplasm within the statocyte perceives gravity resulting in signal transduction and response. Although evidence for both hypotheses can be found throughout the plant kingdom, in higher plants, the starch-statolith model has more experimental support compared to the protoplast-pressure hypothesis. This has come as a result of a plethora of significant studies involving starch-deficient mutants of *Arabidopsis* (Kiss, 2000).

Role of the actin-cytoskeleton in gravitropism

Because of the increasing support for the starch-statolith model for gravity perception, the actin cytoskeleton has been a topic of increasing study for its role in this process. According to Baluška and Hasenstein (1997), there are two models for cytoskeletal-based gravity sensing in the roots of higher plants. They are the unrestrained and restrained gravity sensing models. In unrestrained gravity sensing, the statoliths are not anchored by actin microfilaments, as the filaments are not of sufficient strength to anchor them. Therefore, when the statoliths settle in response to a gravity stimulus, they interact with plasma-membrane based receptors which, in turn, transduce the gravity signal. In restrained gravity sensing, the cytoskeleton is robust enough to anchor the statoliths. Upon settling, the statoliths create tension in microfilaments that are linked directly to putative plasma membrane receptors (ion-channels). The tension causes these ion-channels to open and the signal to be transduced. Recently, a third model has emerged, the tensegrity model, for actin-based gravity perception by Yoder et al. (2001). In this model, amyloplasts are not directly linked to actin-microfilaments, but upon settling and moving through the actin, disrupt the latter. The actin, as in restrained gravity sensing, is linked to putative ion-channel receptors, and disruption causes those receptors to be opened. Thus, the tensegrity model is a compromise between the unrestrained and restrained gravity sensing models (Blancaflor, 2002).

Even with the presence of these three models, the exact nature of gravity perception is not fully understood. Pharmacological studies using the MF-disrupting drugs, latrunculin B and cytochalasins have yielded conflicting results (Blancaflor, 2002). In certain studies, depolymerization of actin with latrunculin B led to the promotion of gravitropism in roots with

decreased statolith movement (Palmieri and Kiss, 2005). This suggested that actin was not necessary for perception, but modulated it by regulating its magnitude and its initiation and completion. Previous studies, (Yamamoto and Kiss, 2002; Yoder et. al, 2001) had found that promotion of gravitropism resulted from increased statolith movement after latrunculin B mediated depolymerization. Taken together, these studies seem to support restrained gravity sensing in one respect, but also lend support to unrestrained gravity sensing in another. However, studies using various mutants of actin-binding proteins have provided some insight into actin's role in gravitropism (Blancaflor, 2002).

Actin-Binding Proteins

Currently, the precise link between actin binding protein effects on the cytoskeleton and gravitropism is unknown (Blancaflor, 2002). Some of the ABP (Actin Binding Proteins) which are involved in reorganizing the cytoskeleton are the profilins, ADF (Actin Depolymerizing Factor), myosins, fimbrins, and villins. These proteins function by increasing the rate of G-actin monomer inclusion into F-actin strands, severing filaments, capping the ends of filaments, or crosslinking (bundling) filaments together (Gibbon, 2001). The profilins principally bind G-actin monomers and in animals, function by interacting with the Actin-Related Protein (ARP) 2/3 complex and the phosphoinositides (specifically inositol 1,4-5 triphosphate) to regulate F-actin polymerization. Both plant and vertebrate profilins have also been shown to have the capacity to polymerize vertebrate actin (Pereloizen et al., 1996; Ballweber et al., 1998, reviewed in Gibbon, 2001).

The effects of ADF on the regulation of actin-dynamics are more complex than profilin's effect (Gibbon, 2001). ADF functions in the phenomenon of actin treadmilling by increasing both the rates of G-actin removal at the pointed (-) end and addition of monomers to the barbed (+) end of the microfilament. The association of plant profilins with ADF increases the treadmilling rate to approximately 3-fold that of ADF alone (Didry et al, 1998). pH has also been found to increase ADF-mediated depolymerization (alkaline pH) and to promote polymerization (alkaline pH). The effect of alkaline pH on increasing dissociation rates is hypothesized to result from increased dissociation at the pointed end (Carlier et al., 1997).

Because changes in cytoplasmic pH in the columella cells of *Arabidopsis* have been reported (Scott and Allen, 1999; Fasano et al., 2001) in the early stages of gravitropism, these studies further support the role of pH changes in regulating the activities of ADF and cytoskeletal organization. Taken together, they suggest a role for ADF and other actin-binding proteins in the gravitropism pathway (Blancaflor, 2002).

Myosins have been implicated in gravitropism as a result of their localization to amyloplasts by immunofluorescence techniques (Blancaflor, 2002). Myosins have also been shown to affect the active transport of amyloplasts (Volkman et al., 1999), and pharmacological studies have demonstrated that the active transport of amyloplasts during gravistimulation is disrupted by the cytocholasins. A previous study by Volkman et al. (1999) suggested that the myosins may also be involved in regulating the salutatory (oscillatory) motions of statoliths in gravitropism, and gravity perception may be perceived in terms of the displacement of statoliths relative to the background saltatory noise (Volkman and Baluska, 1999).

The fimbrins and the villins have been shown to cross link actin filaments (Blancaflor, 2002). Tominaga et al. (2000) suggested that the fimbrins may be involved in bundling actin filaments, and Kovar et al. (2001) showed that the microinjection of fluorescently labeled fimbrins into plant cells created a more dynamic cortical actin framework (Blancaflor, 2002). To date, the role of villins and fimbrins in gravitropism is unknown.

Currently, the effects of cytoskeletal organization on gravitropism cannot be reconciled with the conflicting results of the pharmacological studies (Blancaflor and Masson, 2003). Some have argued that the cytoskeleton may not be involved in mediating the signal transduction process but may be involved in modulating the response (Correll and Kiss, 2002) based on the latrunculin B studies involving the promotion of gravitropism in the midst of cytoskeletal disruption (Staves and others, 1997; Yamamoto and Kiss, 2002). However, others have argued that the enhancement of gravitropism in these studies may be linked to the altered targeting of auxin transporters and, indeed, the cytoskeleton is involved in the signaling process (Blancaflor and Masson, 2003). This is based on pharmacological studies involving the *Arabidopsis PIN-FORMED (PIN1 and PIN2)* genes that encode auxin efflux transporters in

hypocotyls and roots. These studies have shown that PIN1's ability to relocate is F-actin dependent (Geldner et al., 2001). In addition, the auxin uptake carrier, *AUX1*, has been found in columella cells and its localization appears to be altered in some respect by gravity (Swarup et al., 2001). The argument by Blancaflor and Masson (2003) is also based on studies involving the *mdr* mutants (Noh et al., 2003) which mislocalize PIN1 leading to a larger lateral auxin conductance. Therefore, it is intended that this current study on the ARP 2/3 complex should bring some clarity to this issue.

Structure and function of the Arabidopsis ARP 2/3 Complex

The ARP (Actin Related Protein) 2/3 complex (Fig. 1) was first purified from the protist, *Acanthamoeba castellanii*, and is principally named for its two major subunits, actin-related proteins ARP 2 and ARP 3. The complex consists of a total of seven subunits including ARP 2, ARP 3, ARPC1, ARPC2, ARPC3, ARPC4, and ARPC5. The subunits ARPC1-5 were originally named based on size. Since its initial purification, the complex has been purified from yeasts and vertebrates (Goley and Welch, 2006). Although it has not been purified in plants, the discovery of many homologues in *Arabidopsis* to other organisms strongly suggests its existence in higher plants (Mathur, 2005). The putative *Arabidopsis* ARP 2/3 is referred to as actin-related since it was found to be associated with the actin binding protein, profilin (Machesky et al., 1994).

When alone, ARP 2/3 has little biochemical activity and requires association with NPF (nucleation promoting factor) proteins to function. Upon activation, it has the capacity to bind to the sides of existing F-actin filaments and to promote nucleation of new daughter filaments. This ARP 2/3 mediated branching of F-actin occurs with daughter filaments branching at a 70° angle to the original filament, and evidence in support of the side branching model has come from direct observation of ARP 2/3 mediated branching in vitro using fluorescence microscopy (Amann and Pollard, 2001). Genetic studies have demonstrated the requirement of a functioning ARP 2/3 complex for viability in many unicellular and multicellular organisms other than plants. In *Saccharomyces cerevisiae* and *S. pombe*, mutations in genes of any subunit results in effects ranging from severe growth reductions to death of the organism (Goley and Welch, 2006).

In plants, the effects are generally not lethal, but lead to prominent phenotypes in *Arabidopsis* trichomes, hypocotyl, and leaf epidermal cells. Trichomes in mutants tend to be irregularly shaped, and leaf and hypocotyl epidermal cells are misshapen and peel away from each other during cell growth. Primarily because of the misshapen or distorted appearance of trichomes, the putative ARP 2/3 genes identified in *Arabidopsis* were collectively referred to as the *DISTORTED* class of genes (Hulskamp et al., 1994). There are 8 total genes in *DISTORTED*: *ALIEN*, *CROOKED* (ARPC5), *DISTORTED1* (ARP3), *DISTORTED2* (ARPC2), *GNARLED* (NAP135), *KLUNKER* (PIR121), *SPIRRIG*, and *WURM* (ARP2). Because these mutants could be phenocopied by treating wild-type (WT) leaves of *Arabidopsis* with actin-disrupting drugs (cytochalasins, latrunculin B, phalloidin, and jasplakinolide), a mass candidate gene approach was instigated for the cloning of these genes. *CROOKED* was the first to be cloned, followed by *DIS1*, *DIS2*, and *WURM*. However, homologues for three of the ARP 2/3 subunits have been not been conclusively identified, and some of the genes in *DISTORTED* (*GNARLED* and *KLUNKER*) refer to ARP 2/3 activators rather than to the actual subunits of the complex. Homologues for *SPIRRIG* and *WURM* have not been identified to date.

As mentioned previously, the NPFs (nucleation promoting factors) have been identified as the activators of ARP 2/3 in other organisms (Goley and Welch, 2006). *Drosophila melanogaster* and *Caenorhabditis elegans*, contain a SCAR (suppressor of cyclic AMP repressor) complex also known as WAVE (WASP-family verprolin-homologous protein). The inactive form consists of the SCAR1 protein complexed with ABI, HSPC300, NAP125, and PIR121. In one proposed mechanism, activation of the SCAR complex occurs when GTP-bound Rac binds to PIR121, which leads to disassociation of SCAR1 and HSPC300 from the rest of the complex, allowing SCAR1-HSPC300 to activate ARP 2/3 (Millard et al., 2004). The SCAR complex associates to ARP 2/3 via the interaction of SCAR1's VCA (verproline homology connecting acidic domain) region with ARP 2/3 (Eden et al, 2002; Bear et al., 1998). After activation, the SCAR complex disassociates from ARP 2/3 and is available to activate other ARP 2/3 complexes throughout the cytoplasm (Egile et al., 2005).

However, in plants, the regulatory mechanism for ARP 2/3 is somewhat obscure due to the scarcity of homologues. *Arabidopsis* *GNARLED* encodes a NAP125 homologue (El-Assal et

al., 2004) and the *KLUNKER* gene may encode PIR121 (Mathur, 2005) and a new gene, *BRICK1*, may encode the HSPC300 protein. *Arabidopsis ROP2* appears to be the homologue for the Rac GTPase (Basu et al., 2004). Although the entire SCAR complex has not been identified, these studies indicate its presence. It is currently not known whether ARP 2/3 is activated in plants in the same way it is activated in other organisms that contain SCAR/WAVE.

Potential role of ARP 2/3 in gravitropism and phototropism

To date, the ARP 2/3 has never been studied in terms of tropistic curvature in hypocotyls or in roots. Studies so far on ARP 2/3 have been concentrated on its role in cell elongation and in arranging actin microfilaments (El-Assal et al., 2004; Le et al., 2003). This study is focused on two of the *DISTORTED* family of ARP 2/3 mutants, *dis1* (distorted 1) and *dis2* (distorted 2). The phenotypes for *dis1* and *dis2* are similar to wild-type mutants treated with actin disrupting drugs (Schwab et al., 2003; Szymanski et al., 1999). They are characterized by abnormally shaped trichomes, cell-adhesion defects, and a randomly bundled and disorganized array of actin microfilaments. Both *dis* mutants are viable, but do show a reduction in FW (free weight) (El-Assal et al., 2004; Le et al., 2003). Overall, the *dis* mutations do not appear to affect whole-plant architecture and do not cause the severe developmental defects found in other ARP 2/3 mutant organisms (Le et al., 2003).

Results from latrunculin-based actin depolymerization studies in *Arabidopsis* and studies demonstrating the dependence of auxin transporters on an intact F-actin cytoskeleton strongly suggests that an intact and organized cytoskeleton is a requirement in the gravitropic pathway. In addition, since there are many instances where the pathways of phototropism interact with those of gravitropism (Corell and Kiss, 2002), it seems likely that an effect on the cytoskeleton may influence that pathway as well.

The goal of this study was to determine whether the ARP 2/3 plays a role in gravitropism and phototropism. The mutants, *dis1* and *dis2*, have been shown to be ARP 2/3 mutants by cloning and rescue studies in *S. cerevisiae*. These studies suggest that these genes encode *Arabidopsis* ARP 2/3 subunits ATARP3 and ATARPC2 (El-Assal et al., 2004; Le et al., 2003). We

show that both *dis1* and *dis2* affect gravitropism in a light dependent manner and are involved in the phototropism signaling pathway.

Figure 1

An illustration of the structure of ARP 2/3 subunits and the overall function of the ARP 2/3 complex in nucleating F-actin. This illustration is taken from a review by Goley and Welch (2006), and we added the known gene homologues of the individual proteins found in *Arabidopsis* reported in Mathur (2005). They are indicated in parentheses next to the protein that they encode. After assembly, the complex binds to the side of the mother filament at a 70° angle and promotes nucleation of a new daughter filament, *de novo*, by acquiring G-actin monomers.

Figure 1

MATERIALS AND METHODS

Seed source and culture conditions

The wild-type (WT) seeds used in our studies belonged to the Columbia (Col) ecotype of *Arabidopsis thaliana*. The seeds of both *dis1* (*distorted 1*) and *dis2* (*distorted 2*) were created from an ethylmethane sulfonate (EMS) mutagenized population of wild-type (Col) plants (as described by El-Assal et al., 2004 and Le et al., 2003). These seeds were kindly provided by Dr. Danel B. Szymanski (Purdue University, West Lafayette, Indiana, USA).

For both gravitropism and phototropism studies, seeds were surface sterilized in 70% (v/v) ethanol containing 0.002% (v/v) Triton X-100 for 5 min., followed by two 1-min rinses in 95% (v/v) ethanol. Seeds were then washed several times in sterile double distilled water. Seeds were then placed onto sterile cellulose film placed on top of 1.2% (w/v) agar, containing one-half-strength Murashige and Skoog salts with 1% (w/v) sucrose and 1 mM MES at pH 5.5 in square (100 X 15-mm) petri plates. The petri plates were then sealed with Parafilm and placed on their edge so that the surface of agar was vertical and the seedlings were grown for 4 d in either white fluorescent light ($70 \mu\text{mol.m}^{-2}.\text{s}^{-1}$) or in darkness at 23°C.

Gravitropism and phototropism experiments

Both gravitropism and phototropism experiments were conducted in a dark room at 20-22°C. For the gravitropism studies, plates were reoriented at 90 degrees from the vertical. In the phototropism studies, plates from both light and dark treatments were placed vertically and exposed to unidirectional blue light having a fluence rate of $15\text{-}20 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ received through a blue Plexiglas filter (Rohm and Haas No. 2424; transmission maximum 490 nm). Photographs were taken with a digital camera using dim green safe light (fluence rate $< 0.8 \mu\text{mol.m}^{-2}.\text{s}^{-1}$) at 0, 0.5, 1, 2, 4, 8, and 24 h time points. Previous studies have demonstrated that dim green safe light had little to no effect on the tropistic responses of *Arabidopsis* seedlings (Fitzelle and Kiss, 2001).

Growth and curvature analyses

Measurements of growth and curvature of hypocotyls and roots were performed using Image-Pro Plus (version 5.01, Media Cybernetics, Silver Spring, MD). Growth rate was defined as the increase in length from the starting point. Tropistic curvature was defined as the change in angle from the starting point. Gravitropic curvature was defined as positive (curving toward the gravity vector) or negative (curving away from the gravity vector). In phototropism, plant organs turning toward light were assigned a positive value and those turning away from light were assigned a negative value.

In orientation studies of light- and dark-grown seedlings, curvature values were obtained for light- and dark-grown seedlings at the 0 time point, before seedlings were photo or gravistimulated. During time course of curvature studies, curvature was assessed at the 0.5, 1, 2, 4, 8, and 24 h time points, and the change in angle over time from 0 h was measured. Values were reported as the mean change in curvature ($\pm$ SE). In orientation studies, both the mean value and standard deviation (SD) for each genotype were reported and used to determine degree of orientation. Statistical differences in the tropistic responses in time-course and growth studies were determined by an ANOVA followed by a Tukey's test ($P < 0.05$), using SigmaStat software (version 2.03, Systat, Richmond, CA). When criteria for an ANOVA were not met, a Kruskal-Wallis ANOVA on ranks was performed followed by Dunn's method ($P < 0.05$).

RESULTS

In order to study the effects of *dis1* and *dis2* on gravitropic curvature, we performed orientation and time-course of curvature analyses and compared with WT. Although studies of hypocotyl and root orientation relative to the gravity vector do not account for the change in curvature over time, they have been shown to be a good assay for gravisensing in seedlings (Kiss et al., 1997). In this study, mean curvature was used in determining orientation relative to the gravity vector. This analysis was performed on 4-d old seedlings in vertically held petri dishes in darkness before any subsequent reorientation of seedlings took place for time-course-of-curvature studies. Separate studies were performed on dark-grown and light-grown seedlings to determine the effects of light on orientation.

After orientation studies, seedlings were exposed to their respective reorientation and blue-light treatments for further study of time course of curvature. Time-course studies were used to differentiate between the effects of *dis1* and *dis2* on phototropic and gravitropic curvature. In studies of gravitropism, light-grown and dark-grown seedlings were analyzed, while only dark grown seedlings were analyzed in the phototropic studies.

In the orientation studies of dark-grown seedlings (Fig. 2), we found that hypocotyls of *dis2* were disoriented relative to the WT, as determined by the mean, while the effects of *dis1* were intermediate between WT and *dis2* (Fig. 2A). Time-course studies, however, revealed no significant differences ($P>0.05$) in curvature among the three genotypes (Fig. 2B).

In roots, we found that both *dis1* and *dis2* were disoriented relative to WT (Fig. 3A). However, there were no differences in orientation between *dis1* and *dis2* mutants. In addition, time-course studies (Fig. 3B) provided evidence that *dis1* had a deficiency in gravitropic curvature, as *dis1* mutants also showed a significant decrease ($P<0.05$) in positive gravitropic curvature relative to WT at 4, 8, and 24 h time points, but no statistically significant difference was noted in *dis2* ($P>0.05$). In terms of growth rate, there was also no significant difference ($P>0.05$) in hypocotyls of *dis1* and *dis2*, compared to WT (Fig. 4A). In roots, *dis1* showed a significant decrease ($P<0.05$) in growth compared to WT (Fig. 4B). However, *dis1* roots are clearly

impaired in gravitropism, since gravitropic curvature at 24h is impaired by 43.3% (Fig. 3B) while growth is reduced by only 14.7% (Fig. 4B) relative to WT.

In light-grown seedlings (Fig. 5), we found that hypocotyls of *dis1* were most oriented relative to gravity, the WT was the least oriented, and *dis2* exhibited an intermediate position (Fig. 5A). However, since there was unilateral light, this study considered interactions between gravitropism and phototropism, and we consider the studies on dark-grown seedlings a more accurate representation of gravitropism. The time-course analysis yielded no significant difference ($P > 0.05$) between the mutants and the WT (Fig. 5B), except at 0.5 h. The orientation studies of roots (Fig. 6A) showed that *dis2* roots were less oriented relative to WT. However, the orientation as expressed by the mean was similar in all three genotypes. Time course studies in roots (Fig. 6B) also revealed a significant increase ($P < 0.05$) in *dis2* curvature relative to WT at the 0.5h time point, but generally showed no differences at the others.

In light-grown seedlings, growth was significantly diminished ($P < 0.05$) in hypocotyls of *dis1* compared to WT, while *dis2* exhibited no difference (Fig. 7A). There were no significant differences ($P > 0.05$) in the growth of *dis1* or *dis2* roots relative to WT (Fig. 7B).

In order to study the effects of *dis1* and *dis2* mutations on phototropism, we performed time-course of curvature measurements. We found in studies of dark-grown hypocotyls that *dis1* mutants had a significant increase ($P < 0.05$) in phototropic curvature relative to the WT at 4 and 8 h, while *dis2* showed a significant increase ($P < 0.05$) at 4 h (Fig. 8). In roots (Fig. 9), significant ($P < 0.05$) increases in curvature were seen in *dis1* at 1 and 4 h. However, there was no significant difference, overall, ($P > 0.05$) in curvature in *dis1* or *dis2* relative to WT. Growth was not altered in hypocotyls or roots as there was no significant difference ($P > 0.05$) among the three genotypes (Fig. 10).

The effects of *DIS1* and *DIS2* on the tropistic responses of hypocotyls and roots are summarized in Table 1. Based on our results, *DIS1* and *DIS2* have opposite effects on gravitropism. In roots of dark-grown seedlings, *DIS1* promotes gravitropism, while in roots of light-grown seedlings, *DIS2* attenuates gravitropism to a small degree.

In terms of phototropism, the effect of *DIS1* is apparent in both hypocotyls and roots. In hypocotyls and roots of dark-grown seedlings, *DIS1* attenuates phototropism. *DIS2* also attenuates phototropism in hypocotyls, although not in roots.

Figure 2

Orientation (A) and time course of curvature (B) studies of gravitropism in hypocotyls of dark-grown 4-day old seedlings of WT (Columbia ecotype), *dis1* (*distorted1*), and *dis2* (*distorted2*) genotypes of *Arabidopsis thaliana* (Columbia ecotype).

In the orientation study (A), hypocotyls of *dis2* seedlings were most disoriented relative to WT, followed by hypocotyls of *dis1*. In this study the petri plates were kept vertical, and images were taken in darkness before treatment commenced. The relative degree of orientation with the gravity vector in WT, *dis1* and *dis2* hypocotyls is indicated by the mean. The mean was calculated for 144 – 184 seedlings. Values on the horizontal axis (Degrees from the vertical) indicate categories of 10 degree spans of 0 to >70 degrees. The categories are as follows: 0-10°, >10-20°, >20-30°, >30-40°, >40-50°, >50-60°, >60-70°, >70°. The number of seedlings within each category is indicated on the vertical axis.

In the time course of curvature study (B), there was no significant difference ($P > 0.05$) in curvature for *dis1* or *dis2* hypocotyls relative to WT. In this study, the petri plates were reoriented 90° degrees from the vertical and kept in darkness throughout the treatment. Measurements of the change in curvature over time were recorded over a period of 24 h at discrete time points. Mean curvature at each data point was calculated for 87 – 144 seedlings. Error bars represent SE. A statistically significant difference of curvature ($P < 0.05$) at any data point is indicated by a (*) adjacent to the curve.

Figure 2

Figure 3

Orientation (A) and time course of curvature (B) studies of gravitropism in roots of dark-grown 4-day old seedlings of WT, *dis1* (*distorted1*), and *dis2* (*distorted2*) genotypes of *Arabidopsis thaliana* (Columbia ecotype).

In the orientation study (A), roots of *dis1* and *dis2* seedlings were similarly disoriented relative to WT. In this study the petri plates were kept vertical, and images were taken in darkness.

The relative degree of orientation with the gravity vector in WT, *dis1* and *dis2* roots is indicated by the mean. The mean was calculated for 145 - 185 seedlings. Values on the horizontal axis (Degrees from the vertical) indicate categories of 10 degree spans from 0 to >70 degrees. The categories are as follows: 0-10°, >10-20°, >20-30°, >30-40°, >40-50°, >50-60°, >60-70°, >70°.

The number of seedlings within each category is indicated on the vertical axis.

In the time course of curvature study (B), there was a significant decrease ($P < 0.05$) in curvature in *dis1* roots relative to WT at 4, 8, and 24 h. In this study, the petri plates were reoriented 90° degrees from the vertical and kept in darkness throughout the treatment. Measurements of the change in curvature over time were recorded over a period of 24 h at discrete time points.

Mean curvature at each data point was calculated for 45 - 60 seedlings. Error bars represent SE. A statistically significant difference of curvature ($P < 0.05$) at any data point is indicated by a (*) adjacent to the curve.

Figure 3

Figure 4

Growth studies during gravistimulation in hypocotyls (A) and roots (B) of dark-grown seedlings of WT, *dis1* (*distorted1*), and *dis2* (*distorted2*) genotypes of *Arabidopsis thaliana* (Columbia ecotype). In hypocotyls, there was no significant difference ($P>0.05$) in growth among the genotypes. However, in roots (B), *dis1* showed a significant reduction ($P<0.05$) in growth rate compared to WT and *dis2*. In this study, the petri plates were reoriented 90° degrees from the vertical and kept in darkness throughout the treatment. Measurements of growth over time were recorded over a period of 24 h at discrete time points. Mean growth rate at each data point was calculated for 87 - 114 seedlings. Error bars represent SE. A statistically significant difference of growth rate ($P<0.05$) at any data point is indicated by a (*) over the bar.

Figure 4

Figure 5

Orientation (A) and time course of curvature (B) studies of gravitropism in hypocotyls of light-grown 4-day old seedlings of WT, *dis1* (*distorted1*), and *dis2* (*distorted2*) genotypes of *Arabidopsis thaliana* (Columbia ecotype).

In the orientation study (A), hypocotyls of *dis1* were most oriented relative to WT, while hypocotyls of *dis2* were intermediate. In this study the petri plates were kept vertical, and images were taken in darkness. The relative degree of orientation with the gravity vector in WT, *dis1* and *dis2* hypocotyls is indicated by the mean. The mean was calculated for 114 - 150 seedlings. Values on the horizontal axis (Degrees from the vertical) indicate categories of 10 degree spans from 0 to >70 degrees. The categories are as follows: 0-10°, >10-20°, >20-30°, >30-40°, >40-50°, >50-60°, >60-70°, >70°. The number of seedlings within each category is indicated on the vertical axis.

In the time course of curvature study (B), there was no significant difference ($P > 0.05$) in curvature for *dis1* or *dis2* hypocotyls relative to WT, except at 0.5h. In this study, the petri plates were reoriented 90° degrees from the vertical and kept in darkness throughout the treatment. Measurements of the change in curvature over time were recorded over a period of 24 h at discrete time points. Mean curvature at each data point was calculated for 25 - 54 seedlings. Error bars represent SE. A statistically significant difference of curvature ($P < 0.05$) at any data point is indicated by a (*) adjacent to the curve.

Figure 5

Figure 6

Orientation (A) and time course of curvature (B) studies of gravitropism in roots of light-grown 4-day old seedlings of WT, *dis1* (*distorted1*), and *dis2* (*distorted2*) genotypes of *Arabidopsis thaliana* (Columbia ecotype).

In the orientation study (A), roots of both *dis1* and *dis2* were more oriented relative to WT. In this study the petri plates were kept vertical, and images were taken in darkness. The relative degree of orientation with the gravity vector in WT, *dis1*, and *dis2* roots is indicated by the mean. The mean was calculated for 115 - 149 seedlings. Values on the horizontal axis (Degrees from the vertical) indicate categories of 10 degree spans from 0 to >70 degrees. The categories are as follows: 0-10°, >10-20°, >20-30°, >30-40°, >40-50°, >50-60°, >60-70°, >70°. The number of seedlings within each category is indicated on the vertical axis.

In the time course of curvature study (B), there was a significant increase ($P < 0.05$) in curvature of *dis2* roots relative to WT at 0.5 h. However, there was no significant difference ($P > 0.05$) in curvature overall at the other time points. In this study, the petri plates were reoriented 90° degrees from the vertical and kept in darkness throughout the treatment. Measurements of the change in curvature over time were recorded over a period of 24 h at discrete time points.

Mean curvature at each data point was calculated for 27 – 64 seedlings. Error bars represent SE. A statistically significant difference of curvature ($P < 0.05$) at any data point is indicated by a (*) adjacent to the curve.

Figure 6

Figure 7

Growth studies during gravistimulation in hypocotyls (A) and roots (B) of light-grown seedlings of WT, *dis1* (*distorted1*), and *dis2* (*distorted2*) genotypes of *Arabidopsis thaliana* (Columbia ecotype). In hypocotyls (B), *dis1* showed a significant reduction ($P < 0.05$) in growth rate compared to WT and *dis2*. In roots, however, there was no significant difference ($P > 0.05$) in growth among the genotypes. In this study, the petri plates were reoriented 90° degrees from the vertical and kept in darkness throughout the treatment. Measurements of growth over time were recorded over a period of 24 h at discrete time points. Mean growth rate at each data point was calculated for 31 - 47 seedlings. Error bars represent SE. A statistically significant difference of growth rate ($P < 0.05$) at any data point is indicated by a (*) over the bar.

Figure 7

Figure 8

Time course of curvature studies of phototropism in dark-grown, hypocotyls of 4 day-old seedlings of WT, *dis1*, and, *dis2* genotypes of *Arabidopsis thaliana* (Columbia ecotype). *Dis1* mutants had a significant ($P<0.05$) increase in curvature relative to WT at 4, and 8 h. *Dis2* also had a significant increase ($P<0.05$) in curvature at 4 h. In this study, the petri plates were placed vertically and plants were exposed to unidirectional blue light ($15\text{-}20\ \mu\text{mol.m}^{-2}.\text{s}^{-1}$) throughout the treatment. Measurements of the change in curvature over time were recorded over a period of 24 h at discrete time points. Mean curvature at each data point was calculated for 45 - 52 seedlings. Error bars represent SE. A statistically significant difference of curvature ($P<0.05$) at any data point is indicated by a (*) adjacent to the curve.

Figure 8

Figure 9

Time course of curvature studies of phototropism in dark-grown, roots of 4 day-old seedlings of WT, *dis1*, and, *dis2* genotypes of *Arabidopsis thaliana* (Columbia ecotype). *Dis1* mutants had a significant ($P<0.05$) increase in curvature relative to WT at 1, and 4 h, while *dis2* showed no significant differences ($P>0.05$) in curvature. In this study, the petri plates were placed vertically and plants were exposed to unidirectional blue light ($15\text{-}20\ \mu\text{mol.m}^{-2}.\text{s}^{-1}$) throughout the treatment. Measurements of the change in curvature over time were recorded over a period of 24 h at discrete time points. Mean curvature at each data point was calculated for 24 - 36 seedlings. Error bars represent SE. A statistically significant difference in curvature ($P<0.05$) at any data point is indicated by a (*) adjacent to the curve.

Figure 9

Figure 10

Growth studies during phototropic studies in hypocotyls (A) and roots (B) of dark-grown seedlings of WT, *dis1* (*distorted1*), and *dis2* (*distorted2*) genotypes of *Arabidopsis thaliana* (Columbia ecotype). In both hypocotyls (A) and roots (B), there was no significant difference ($P>0.05$) in growth among the genotypes. Measurements of growth over time were recorded over a period of 24 h at discrete time points. In these studies, the petri plates were placed vertically and plants were exposed to unidirectional blue light ($15\text{-}20\text{ }\mu\text{mol.m}^{-2}.\text{s}^{-1}$) throughout the treatment. Mean growth rate at each data point was calculated for 31 - 47 seedlings. Error bars represent SE. A statistically significant difference of growth rate ($P<0.05$) at any data point is indicated by a (*) over the bar.

Figure 10

Table 1

The effects of *DIS1* and *DIS2* on the tropistic responses of hypocotyls and roots are summarized in Table 1. Based on our results, *DIS1* and *DIS2* have opposite effects on gravitropism. In roots of dark-grown seedlings, *DIS1* promotes gravitropism, while in roots of light-grown seedlings, *DIS2* attenuates gravitropism to a small degree.

In terms of phototropism, the effect of *DIS1* is apparent in both hypocotyls and roots. In hypocotyls and roots of dark-grown seedlings, *DIS1* attenuates phototropism. *DIS2* also attenuates phototropism in hypocotyls, although not in roots.

The positive and negative signs in this table indicate statistically significant ($P < 0.05$) differences in response as determined by the data in previous figures.

Table 1- Summary of the effects of *DIS1* and *DIS2* on tropistic responses compared to WT.

Tropism	Growth Conditions	Organ	Method	<i>DIS1</i>	<i>DIS2</i>
Gravitropism	Dark	Hypocotyl	Orientation	O	O
			Time-Course of Curvature	O	O
		Root	Orientation	O	O
			Time-Course of Curvature	+	O
	Light	Hypocotyl	Orientation	O	O
			Time-Course of Curvature	O	O
		Root	Orientation	O	O
			Time-Course of Curvature	O	-
Phototropism	Dark	Hypocotyl	Time-Course of Curvature	-	-
		Root	Time-Course of Curvature	-	O

DISCUSSION

DIS1 and DIS2 play a role in gravitropism in roots.

Based on studies of dark- and light-grown seedlings, we propose that *DIS* (*DISTORTED*) 1 and *DIS2* affect gravitropism in roots but not hypocotyls (Table 1). In roots of dark-grown seedlings, *DIS1* appears to play a strong role in promoting gravitropism, since roots of *dis1* were strongly impaired in gravitropic curvature beyond 4 h in the time-course study (Fig. 3B). In roots of light-grown seedlings, *DIS2* plays a role in attenuating gravitropism, although this role may be weak. Although mutants of *dis2* had a significant ($P<0.05$) enhancement in gravitropic curvature relative to WT in the time-course study, the increase in curvature was small, and was only revealed by the ANOVA test (Fig. 6B) at 0.5 h. It is unlikely that either *DIS1* or *DIS2* affect gravitropism in hypocotyls, since no effects on gravitropism were observed in time-course studies (Table 1). Since the *DIS* mutants have altered ARP 2/3 proteins, our studies suggest that ARP 2/3 is involved with gravitropism.

One possible mechanism by which ARP (ACTIN RELATED PROTEIN) 2/3 interacts in gravitropism signaling pathway is by association with ROP2 (RHO OF PLANTS) and ARFs (AUXIN RESPONSE FACTORS) (Boutte et al., 2007; Xu and Scheres., 2005), ultimately affecting auxin transport in the root. The Cholodny-Went hypothesis, proposed in 1928, implicates the hormone, auxin, as a necessary factor for gravitropic curvature. In this model, auxin enters the root and is redistributed laterally toward the bottom-half of the root. This asymmetric distribution of auxin, results in gravitropic curvature occurring in the direction away from the half with the highest concentration of auxin (Blancaflor and Masson, 2003).

When auxin enters the gravity perceiving cells of the root cap, many auxin-induced genes are upregulated. Genes for 25 of the AUXIN RESPONSE FACTORS involved in regulating these genes have been identified, and five of them have been shown to be activators of auxin-induced genes. One of these activators, ARF7 has been implicated in the regulation of gravitropism (Leyser, 2006). In previous studies, *arf7* mutants experienced impaired hypocotyl

gravitropism (Liscum and Reed, 2002; Correll and Kiss, 2002). However, other ARF's may be involved in gravitropism as suggested in Geldner et al. (2001).

One possible way in which the ARP 2/3 complex is involved in gravitropism is in the signal transduction pathway downstream of the ARF's, through interacting with ROP2. ROP2 has been found to be responsible for activating ARP 2/3 by binding to the SCAR (SUPPRESSOR OF CYCLIC AMP REPRESSOR) complex in *Arabidopsis* (Basu et. al., 2004), and ROP2 has been linked to the ARF's in previous studies (Nebenführ et al., 2002; Molendijk et al., 2001; Steinman et al., 1999). Steinman et al. (1999) implicated a particular ARF, ARF-GEF (GNANINE NUCLEOTIDE EXCHANGE FACTOR) GNOM in the localization of PIN (PIN-FORMED) auxin efflux carriers. They found that PIN1 carriers were aberrantly localized in *gnom* mutants. ARP 2/3 activation may, in turn, affect gravitropic signal transduction by altering PIN localization and the flow of auxin in the root.

Further study will be needed to confirm the interaction of ARP 2/3 in this pathway. Molendijk et al. (2001) found that ROP2 activity was dependent on actin-mediated vesicle trafficking was regulated by the ARF's, but there has been no direct link between ROP2 and gravitropism in any of the studies to date. However, since we found an effect on gravitropism in ARP 2/3 mutants *dis1* and *dis2*, the possibility exists that ROP2 may be involved. In addition, ROP2 may affect other actin-binding proteins in addition to ARP 2/3, since dark-grown roots of *dis1* were not completely impaired in gravitropism and *dis2* had no effect in dark-grown roots (Fig. 3B, Table 10). Further molecular studies on the substrates of ROP2 may be the key in linking the actin-binding proteins to the signal transduction pathway in gravitropism.

Our results in the gravitropism studies also suggest that *DIS1* and *DIS2* may contribute differently to effects on gravitropism. We found that *DIS1* had a strong effect on gravitropism in the dark (Fig. 3B) while a much weaker effect in *DIS2* was observed in the light (Fig. 6B). *DIS1* had no effect in the light (Table 10). This may be related to the observation that ARP 2/3 mutants have different phenotypes in actin organization observed in various studies. Actin disorganization in ARP 2/3 mutants has been described in numerous microscopy studies. Together, these studies have primarily reported randomly localized dense aggregates of F-actin, highly bundled F-actin and randomly located cortical actin patches (Mathur, 2005). The most

severe phenotype in actin organization appeared in *crk* (*crooked*) trichomes, while the least severe one occurred in *dis2* trichomes (Mathur, 2005). Studies have shown that *dis1* trichomes exhibit aggregation of actin as they elongate, and increased F-actin bundling was seen at the tips of the rapidly growing trichomes (Mathur et al., 2003). However, the exact difference in actin organization among the genotypes is unresolved because of subjective interpretations of disorganization in these studies.

The fact that *DIS1* and *DIS2* promote gravitropism in the dark while they attenuate gravitropism in the light and the difference in the intensity of the effects we observed may also be due to differential expression patterns of the ARP 2/3 protein in light versus darkness. In terms of spatial expression patterns, Klahre and Chua (1999) found differential GUS (BETA-GLUCURONIDASE)-ARP2 staining patterns in roots and inflorescence stems. They also found a difference in expression in light-grown versus dark-grown tissues. Specifically, they found that mRNA transcription was predominately located in vascular tissue in mature inflorescence stems and in the xylem of roots. The conclusion of this study was that *ARP2* was expressed predominately in rapidly growing pollen and in developing xylem vessels, and expression was reduced in mature cells. To date, no studies have analyzed *DIS1* or *DIS2* tissue-specific expression in gravisensing statocytes of *Arabidopsis*. Most of the studies have focused on the cells that are most sensitive to ARP 2/3 mutations, such as trichomes and leaf epidermal cells (Smith and Oppenheimer, 2005). However, it would be interesting to see how ARP 2/3 expression varies among different cell types and how it affects actin organization in statocytes. There are no reports, to date, of gravitropism studies on the other proteins that regulate actin polymerization or on other ARP 2/3 mutants in *Arabidopsis*. Further analysis of expression levels during curvature may suggest that these proteins have differing effects on gravitropism.

DIS1 and DIS2 are involved in phototropism

We did find that in hypocotyls and in roots of dark-grown seedlings, *DIS1* was involved in attenuating phototropism. The explanation for this may also lie in the elucidation of the pathway involving the AUXIN RESPONSE FACTORS and ARP 2/3. Currently, ARF7 has been found to be the link between the phototropism and gravitropism signaling pathways in

Arabidopsis (Correll and Kiss, 2002). However this study suggests that if ARP 2/3 resides in this ARF mediated pathway, other ARF's may be involved. The only possible link of ARP 2/3 to ARF would be the ROP2 protein, based on previous studies (Nebenführ et al., 2002; Molendijk et al., 2001; Steinman et al., 1999). Therefore, future studies characterizing the effects of ROP2 on phototropism are needed to conclusively link ARP 2/3 into the phototropism signaling pathway. Double mutants using *rop2* and the ARP mutants may help to resolve this issue.

In most of our gravitropism and phototropism studies, statistical differences in time course studies did not occur throughout the time frame of the experiments. For example, in the study of gravitropism in roots of light-grown seedlings, only one significant difference in curvature occurred (Fig. 6B). Higher-resolution methods may aid in resolving this complex scenario, where many actin-binding proteins could be involved, making their individual contributions small. For example, ROTATO (a feedback system) has been shown to be effective in determining the subtleties of the kinetics of roots gravitropism and phototropism (Kiss et al., 2003). These studies may further reveal the contributions of *dis1* and *dis2* in gravitropism in roots.

Phototropism and gravitropism are regulated in opposing manners

While comparing the effects of phototropism and gravitropism in roots of dark-grown seedlings, we found that *DIS1*, specifically, had opposite effects on gravitropism and phototropism. *DIS1* promoted gravitropism, while at the same time, attenuated phototropism (Table 1). One hypothesis to explain these observations is that ARP 2/3 may affect translocation of auxin transporters or other actin associated proteins involved in gravitropism and phototropism.

Currently, the PIN (PIN-FORMED) proteins have been implicated in the control of auxin efflux out of cells in *Arabidopsis*. Auxin influx is generally passive, although influx transporters have been identified. Auxin normally enters the cell in a polar, protonated state. The increased pH of the cell converts auxin into a basic, non-protonated state and subsequently makes it non-polar and unable to leave the cell passively. In order to leave the cell, it must be actively transported out via transport proteins (Leyser, 2006).

Three of the six known PINs in *Arabidopsis*, PIN1, PIN2, and PIN3, have been implicated in gravitropism, and their localization has been linked to the actin cytoskeleton. PIN1 has been shown to be dependent on actin-mediated vesicular transport (Geldner et al., 2001), as treatment with brefeldin A (BFA) interferes with PIN1 cycling, and causes PIN1 to accumulate in intracellular “BFA compartments” (Benamins et al., 2005). To date, the potential actin-binding proteins involved in vesicular transport are known. However, it has been demonstrated that transport of vesicles is related to the functions of different proteins, and that these may be related to interaction with the F-actin framework. For instance, depolymerization of actin with cytochalasin D results in the absence of BFA-induced PIN1 accumulation (Geldner et al., 2001), suggesting that an intact F-actin cytoskeleton is responsible for its targeting. In addition, a report by Blakeslee et al. (2004) observed a random distribution of PIN1 in blue light treated dark-grown hypocotyls, similar to what was observed in *mdr* (multidrug resistance gene) 1 mutants which mislocalized PIN1 and experienced a 30% increase in phototropism (Noh et al., 2003). Thus, there may be a link among ARP 2/3 proteins and auxin in transport and in the tropisms, since ARP 2/3 is involved in affecting actin organization. This change in organization may alter the direction of PIN vesicular transport and PIN localization in general, which could result in differences in lateral auxin flow.

It would be interesting to determine the extent of effects of ARP (Actin Related Protein) 2/3 mutations on the localization of PIN proteins during the gravitropic or phototropic response. However, in order to possibly obtain a stronger phenotypic effect in actin organization, one may want to consider characterizing the *crk* (*crooked*) single mutant or a double or triple ARP 2/3 mutant in terms of tropistic responses. Alternatively, multiple mutations involving ARP 2/3 proteins and one or more PIN transporters may help to narrow down the contributions that these transporters make overall to gravitropism or phototropism. Many PINs have been found to be overlapping and redundant in function, and when one PIN is lost, others in the family can change location and compensate for that loss (Vieten et al., 2005). Therefore, the system has to be significantly disabled before significant phenotypes would be expected (Vieten et al., 2005).

We also observed a weak attenuation of phototropism and the promotion of gravitropism in *DIS1* roots of dark-grown seedlings. This suggests that there may be a combined gravitropic and phototropic effect occurring in roots. Therefore, higher resolution studies with *dis1dis2* double mutants or multiple ARP mutants may help to determine the individual contributions of light and gravity in these mutants. It would also be interesting to determine to what extent red and blue light alter phototropism in these mutants and whether the phototropic effect would be enhanced in *dis1dis2* double mutants. More studies on the phototropic and gravitropic effects of PIN and other F-actin associated proteins combined with localization studies may aid in resolving these issues.

Role of the actin cytoskeleton in gravitropism and phototropism

Overall, the results of this study add to the support for a model of actin-cytoskeletal involvement in gravitropism (Blancaflor and Masson 2003). When this study is combined with previously mentioned studies linking altered targeting of auxin transporters to F-actin, it suggests a role for the cytoskeleton in the trafficking of these proteins to their correct location on the plasma membrane. This leads to a hypothesis that light and gravity may influence actin organization via signaling through ARP 2/3 and other actin-monomer binding proteins that regulate polymerization. Since many of the proteins involved in auxin transport are dependent on vesicle trafficking (Boutte et al., 2007), it seems plausible that alterations in the transport network would disrupt the ability of transporters of auxin to reach their correct position in the plasma membrane. We have determined that ARP 2/3 does play a role in gravitropism, and previous studies have demonstrated the contribution of ARP 2/3 in actin organization in various tissues in *Arabidopsis*. Future gravitropic and phototropic studies using mutants of other proteins involved in actin organization may help to resolve issues in the mechanisms of gravitropism and phototropism.

Examples of recent studies which support the role of an intact actin cytoskeleton on gravity perception and in signal transduction have come from ARG (ALTERED RESPONSE TO GRAVITY) mutants (Harrison and Masson, 2008; Kumar et al., 2008). The study by Kumar et al. (2008) suggested a role for ARG1 in amyloplast sedimentation kinetics (gravity perception) and

subsequently gravitropic curvature. In addition, Harrison and Masson (2008) reported mislocalization of PIN3 in hypocotyls of *arg1* and *arl2* (ARG1-LIKE2) mutants; the most severe reduction in gravitropic curvature, though, was found in hypocotyls of *arg1pin3* double mutants. This report suggested that *ARG1* modulated PIN3 trafficking and these affect auxin transport and gravitropism. Thus, our present study suggests that the ARP 2/3 complex may affect gravitropism through the PIN3 auxin transport protein and PIN's in general. However, the phenotypes we observed in gravity perception were primarily noticeable in roots of dark-grown seedlings, and weak in roots grown in the light (Table 10). When taken together with the expression studies of ARP2 by Klahre and Chua (1999), our study suggests that the phenotypes of PIN localization in ARP 2/3 mutants would be small and possibly beyond light microscopy resolution. Therefore, higher resolution transmission electron microscopy may be needed to resolve these subtle phenotypes.

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