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DIS1 and *DIS2* play a role in tropisms in *Arabidopsis thaliana*

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ABSTRACT

To better understand the role of the cytoskeleton in tropisms, we performed studies of gravitropism and phototropism with seedlings of *distorted1* (*dis1*) and *distorted2* (*dis2*) mutants of *Arabidopsis thaliana* which are defective in the ARP (*a*ctin *r*elated *p*rotein) 2/3 complex. The aim of this investigation was to test the hypothesis that this actin-binding protein family is involved in mechanisms of tropisms in plants. In general, we found that *DIS1* has a greater effect on tropisms compared to *DIS2*. *DIS1* enhanced gravitropism in roots of dark-grown seedlings and in inflorescence stems while *DIS2* also enhanced gravitropic responses in inflorescences. In contrast, in blue-light-based phototropism, *DIS1* attenuated the response in hypocotyls of dark-grown seedlings and in red-light-based positive phototropism in roots. Taken together, these studies are the first to suggest that the ARP 2/3 complex, a major family of actin-binding proteins, is involved in the pathways of gravitropism and phototropism.

1. Introduction

Gravity is a constant, ubiquitous force that has influenced plant development throughout 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.

Gravitropism can be divided into the following three processes: perception, signal transduction, and response (Kiss, 2000). Gravity perception occurs in the central columella cells within the root cap, and, in stems and hypocotyls occurs in the endodermal cells, surrounding the vascular cylinder, by a statolith-based mechanism. 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. Numerous studies have suggested that the actin cytoskeleton is involved in the cellular mechanisms of gravitropism from the perception to the response phase (e.g., Baluška and Hasenstein, 1997; Yoder et al., 2001; Blancaflor and Masson, 2003; Kumar et al., 2008b).

Phototropism is the direct growth of a plant toward light, and the phases of this tropism can be defined in a manner similar to gravitropism (Whippo and Hangarter, 2006). In general, the shoots and roots of flowering plants respond to unilateral blue illumination and sensing occurs via the phototropin family of photoreceptors (Lin, 2002). However, directional red-light-sensing has been characterized in roots of *Arabidopsis thaliana*, and this photoresponse is mediated by the phytochrome family of photoreceptors (Kiss et al., 2003; Kumar and Kiss, 2007; Kumar et al., 2008a). The plant hormone auxin is involved in the response phase of phototropism as well as gravitropism, and the actin cytoskeleton mediates auxin transport (Boutté et al., 2007). Several actin-binding protein families, most notably the myosins, have been shown to be involved in the mechanisms of gravitropism (Blancaflor, 2002). To date, one interesting class of actin-binding proteins that have not been assayed for their role in tropisms is the ARP (*a*ctin *r*elated *p*rotein) 2/3 complex (Goley and Welch, 2006). ARP 2/3 mediates branching of F-actin with daughter filaments branching at a 70° angle to the original filament (Amann and Pollard, 2001). Although the ARP 2/3 complex has not been purified in plants, many homologues of this complex have been found in *Arabidopsis* (Mathur, 2005). In addition, a recent study demonstrated ARP3 is localized to actin-nucleating sites in vivo in tobacco cell lines (Maisch et al., 2009).

In plants, the effects of a mutation in the ARP 2/3 complex lead to prominent phenotypes in *Arabidopsis* trichomes, hypocotyl, and leaf epidermal cells (Hülskamp et al., 1994). Primarily because of the misshapen or distorted appearance of trichomes, the putative ARP 2/3 genes identified in *Arabidopsis* are collectively referred to as the *DISTORTED* class of genes. The present study is focused on the analysis of the tropistic behavior of two of the *DISTORTED* family of ARP 2/3 mutants, *dis1* (*distorted 1*) and *dis2* (*distorted 2*). The phenotypes for *dis1* and *dis2* mutants are similar to wild-type plants treated with actin disrupting drugs (Schwab et al., 2003; Szymanski et al., 1999) in that these mutants are characterized by abnormally shaped trichomes, cell-adhesion

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defects, and a randomly bundled and disorganized array of actin microfilaments.

The goal of this study was to test the hypothesis that the ARP 2/3 actin-binding protein family is involved in the cellular mechanisms of tropisms in plants. Thus, we demonstrate that both *dis1* and *dis2* mutants have alterations in gravitropism and phototropism in both seedlings and mature plants. This is the first report to suggest that another key family of actin-binding proteins is involved in the cellular mechanisms of these tropisms.

2. Materials and methods

2.1. Seed source and culture conditions

The wild-type (WT) seeds used in our studies were of the Columbia (Col) ecotype of *A. thaliana*. The seeds of both *dis1 (distorted 1)* and *dis2 (distorted 2)* mutants 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. Daniel B. Szymanski (Purdue University, West Lafayette, Indiana, USA).

For both gravitropism and phototropism studies with seedlings, seeds were first 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 mm × 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 μmol m^{-2} s^{-1}) from above or in darkness at 23 °C.

For gravitropism and phototropism studies with inflorescence stems, seeds were surface sterilized as described above. Seeds were then sown in 10 cm × 10 cm × 8.5 cm plastic pots filled with Metro-Mix 360 (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) as described by Kumar and Kiss (2006). The pots were covered with transparent plastic square plates and placed in plastic trays with continuous white light generated from 34 W fluorescent bulbs at fluence of 70 μmol m^{-2} s^{-1}. When the inflorescence stems were 6–12 cm long (i.e., 20–40 d after sowing, depending on genotype), plants were used for experiments.

2.2. Gravitropism and phototropism experiments

Both gravitropism and phototropism experiments were conducted at 20–22 °C. For the gravitropism studies, plates or pots were reoriented at 90° from the vertical in darkness. In the phototropism studies, plates or pots were placed vertically and exposed to continuous, unidirectional blue light (90° from the vertical) having a fluence rate of 15–20 μmol m^{-2} 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 μmol m^{-2} s^{-1}) at 0, 0.5, 1, 2, 4, 8, and 24 h time points. Previous studies have demonstrated that this dim green safe light had no measurable effect on the tropistic responses of *Arabidopsis* seedlings (Fitzelle and Kiss, 2001).

2.3. Root phototropism experiments with the feedback system

Seedlings were grown in the light as described above, except that smaller round (60 mm × 15 mm) Petri dishes were used in these studies. A computer feedback system, as described by Mullen et al. (2000) and modified by Kiss et al. (2003), was used to constrain the root tip angle to the vertical (parallel to the root axis) during continuous, unilateral red-light (660 nm from an LED) stimulation of 10–20 μmol m^{-2} s^{-1} at 90° relative to the root axis. A root of a seedling was positioned in the center of a rotatable vertical stage (catalog no. RT3S17, Nutec Components, Deer Park, NY) with individual steps of the motor corresponding to 0.17°. The stage was connected to a PC computer, and the stepper motor was controlled by custom software. Roots were imaged every 45 s using infrared illumination (940 nm LED, Radio Shack) and a CCD camera. The software analyzed the images, and if the root tip deviated from 0° (vertical), the software activated the stepper motor to the rotating stage to constrain the tip segment of the root to remain at the vertical. The software recorded the rotation of the stage, and this value is termed "rotation" in Fig. 6.

2.4. 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). The 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 responding toward light were assigned a positive value, and those responding away from light were assigned a negative value.

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 are reported as the mean change in curvature (±S.E.). 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 ($P < 0.05$). In studies using the feedback system, statistical differences were analyzed using least squares regression.

3. Results

In order to study the effects of the *dis1* and *dis2* mutations on gravitropic and phototropic curvature, we performed a time-course of curvature analysis and compared the responses and behavior of the mutants relative to the WT. In studies of gravitropism, light-grown and dark-grown seedlings and inflorescence stems of mature plants were analyzed. In the phototropic studies, light- and dark-grown seedlings and inflorescence stems were analyzed under unidirectional blue light, and a feedback system was used to study root-tip curvature in response to unidirectional red-light stimulation.

In gravitropism experiments of hypocotyls of dark-grown seedlings, time-course studies revealed no significant differences ($P > 0.05$) in curvature among the three genotypes (Fig. 1A). However, in roots, there was a significant decrease ($P < 0.05$) in curvature in *dis1* mutant roots relative to WT at 4, 8, and 24 h, while *dis2* mutants showed no significant ($P > 0.05$) difference (Fig. 1B). In terms of growth rate, there was also no significant difference ($P > 0.05$) in hypocotyls of *dis1* and *dis2* compared to WT (Table 1). However, in roots, *dis1* showed a significant decrease ($P < 0.05$) in growth compared to WT (Table 1). Roots of *dis1* are clearly impaired in gravitropism since gravitropic curvature at 24 h is impaired by 43% (Fig. 1B) while growth is reduced by only 15% (Table 1) relative to WT.

In the gravitropism studies in light-grown seedlings, we again found that in hypocotyls, there were no significant differences