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Pedicel development in *Arabidopsis thaliana*: Contribution of vascular positioning and the role of the BREVIPEDICELLUS and ERECTA genes

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Abstract

Although the regulation of *Arabidopsis* floral meristem patterning and determinacy has been studied in detail, very little is known about the genetic mechanisms directing development of the pedicel, the short stem linking the flower to the inflorescence axis. Here, we provide evidence that the pedicel consists of a proximal portion derived from the young flower primordium, and a bulged distal region that emerges from tissue at the bases of sepals in the floral bud. Distal pedicel growth is controlled by the KNOTTED1-like homeobox gene *BREVIPEDICELLUS* (*BP*), as *35S::BP* plants show excessive proliferation of pedicel tissue, while loss of *BP* conditions a radial constriction around the distal pedicel circumference. Mutant radial constrictions project proximally along abaxial and lateral sides of pedicels, leading to occasional downward bending at the distal pedicel. This effect is severely enhanced in a loss-of-function *erecta* (*er*) background, resulting in radially constricted tissue along the entire abaxial side of pedicels and downward-oriented flowers and fruit. Analysis of pedicel vascular patterns revealed biasing of vasculature towards the abaxial side, consistent with a role for *BP* and *ER* in regulating a vascular-borne growth inhibitory signal. *BP* expression in a reporter line marked boundaries between the inflorescence stem and lateral organs and the receptacle and floral organs. This boundary expression appears to be important to prevent homeotic displacement of node and lateral organ fates into underlying stem tissue. To investigate interactions between pedicel and flower development, we crossed *bp er* into various floral mutant backgrounds. Formation of laterally-oriented bends in *bp lfy er* pedicels paralleled phyllotaxy changes, consistent with a model where the architecture of mutant stems is controlled by both organ positioning and vasculature patterns. Collectively, our results indicate that the *BP* gene acts in *Arabidopsis* stems to confer a growth-competent state that counteracts lateral-organ associated asymmetries and effectively radializes internode and pedicel growth and differentiation patterns.

Keywords: Stem; Homeobox; Kinase; Sepal; Architecture; Node; Internode; Radial symmetry; Vascular bundle

Introduction

Morphogenesis in multicellular organisms is orchestrated by factors that activate cell growth and division, as well as by other factors acting antagonistically to attenuate these processes. Asymmetries in organ shape often arise from differential activation of growth as a result of biased exposure to signaling molecules. Superimposed on growth control is differentiation, wherein cell fate is influenced by signaling from neighboring cells as well as distantly produced regulators. A fundamental aspect of development in multi-cellular organisms is the coordination of patterns of differentiation and growth to derive functional and adaptive organs and physiologies.

In animals, the basic body plan is usually established during embryogenesis, whereas most plants produce new organs throughout the adult stage of the lifecycle. All organs arise from groups of pluripotent stem cells, termed meristems, which exist at the apices. In the shoot apical meristem (SAM), where founder cells for all aerial organs are produced, growth is governed by a number of positive and negative regulators of stem cell identity and division. The WUSCHEL (WUS) homeodomain protein promotes stem cell fate, whereas members of the CLAVATA family inhibit WUS to limit growth (Fletcher, 2002). In contrast,

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proteins encoded by the KNOX gene family promote meristem development by inducing proliferation of pre-specified stem cells.

In addition to their role in meristems, KNOX genes also influence architectures of determinate organs by promoting cell division and delaying differentiation. For example, constitutive activation of the *KNOX* gene *BREVIPEDICELLUS* (*BP*) induces lobing and ectopic meristem formation at margins of *Arabidopsis* leaves (Lincoln et al., 1994; Chuck et al., 1996), indicative of prolonged activity of the leaf marginal meristem (Hagemann and Gleissberg, 1996). This effect is magnified in tomato compound leaves expressing *KNOTTED1*, where the number of leaflets per compound leaf is increased from 5 to 7 to greater than 2000 (Hareven et al., 1996). In *Antirrhinum*, spontaneously arising mutations that cause ectopic expression of *KNOX* genes in petals lead to formation of petal spurs, a feature found in flowers of related taxa (Golz et al., 2002). Mutant spurs sprout from ventral petals as localized growths resembling petal tubes, suggesting that *KNOX* gene-induced growth competence can co-operate with existing developmental states to generate novel sites of growth and morphological variation. When ectopically expressed in female organs of Arabidopsis, the *KNOX* gene *KNAT2* also promotes excess growth, inducing homeotic transformations of ovule nucelli into carpels (Pautot et al., 2001) and again illustrating the consequences of imposing indeterminacy on a normally determinate organ in a specific developmental framework (carpel development). Taken together, the available data indicate that *KNOX* genes confer a meristematic state upon plant tissues in a variety of morphogenetic contexts, making the gene family a potentially versatile tool to mediate evolutionary transformations.

In contrast to SAMs, where balanced growth maintains a population of stem cells throughout development, floral meristems are determinate due to repression of growth following the initiation of sex organs. In *Arabidopsis*, floral meristem identity requires the *LEAFY* (*LFY*) transcription factor, as strong *lfy* mutants display indeterminate floral meristems, a switch in floral phyllotaxy from whorled to helical and transformation of floral organs into leaves (Schultz and Haughn, 1991; Weigel et al., 1992). Additionally, the pedicel, a short specialized internode that links the flower to the inflorescence stem, elongates to form an axillary stem complete with cauline leaves and bract. Although a great deal is known of the molecular genetic mechanisms that act downstream of *LFY* to govern the position, identity and patterning of floral organs, there is virtually no information on the processes that distinguish pedicels from other types of stems.

In many plant species, a defining feature of the pedicel is a bulge at the receptacle region where floral organs attach. An example is in cactus flowers where the ovaries are embedded within an enlarged distal pedicel that protects the developing embryos and seeds (Boke, 1980). Previously, we showed that the KNOX factor BP and the receptor protein kinase ERECTA are required for pedicel growth in *Arabidopsis*. In *bp er* double mutants, pedicels bend downward and have reduced stomata, intercellular spaces and chlorenchyma on abaxial (ventral; see Fig. 1A) sides. Here, we extend these findings by demonstrating that *BP* is also necessary for establishment of a distal pedicel bulge and sufficient to promote its excess growth. We provide evidence that induction of growth at lateral organ/stem junctions is necessary to counteract growth inhibitors associated with lateral organs and vasculature and thereby define the radial architecture of stems.

Methods

Histology and microscopy

Sections (20–30 μm) for fluorescence microscopy and GUS staining were prepared using a vibratome (Leica VT1000S) from fresh leaf or stem tissue embedded in 4% to 6% agar. For fluorescent imaging of chlorophyll and vasculature, vibratome sections were immediately mounted in 50% glycerol and visualized with a Zeiss Axiophot microscope and a Curtis ebq 100 fluorescent lamp. For *BP::GUS* transgenic tissue, sections were placed directly in GUS histochemical staining solution (800 μl of GUS staining buffer [100 mM Na_2PO_4 pH 7.0, 0.5 mM $K_3Fe_3(CN)_6$, 0.5 mM $K_4Fe_2(CN)_6$, 10 mM EDTA, 0.1% Triton X-100], 200 μl of methanol and 12 μl of 50 mg/ml X-gluc [5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid cyclohexyl-ammonium salt] dissolved in dimethylformamide) and incubated for varying times at room temperature or 37°C. After staining, tissue was rinsed twice with 70% ethanol to stop the reaction, fixed for 10 min in FAA and then cleared by passing sections through a graded ethanol series. Sections were rehydrated, mounted in 50% glycerol and viewed and photographed with a Zeiss Axiophot microscope and digital imaging system. For toluidine blue staining, thin sections were prepared as described in Douglas et al. (2002). Vibratome-generated sections were fixed for 30 min in FAA, washed twice with water, incubated for 30 s in 0.05% toluidine blue dissolved in 0.1 M sodium carbonate, rinsed twice with water and then viewed in 50% glycerol. Tissue for dark field microscopy was prepared by fixing in cold FAA overnight, followed by dehydration through a graded ethanol series. Tissue was rehydrated to 70% ethanol and then cleared and mounted in a mixture of chloral hydrate/glycerol/water (8:2:1). Scanning electron microscopy was performed as described in Douglas et al. (2002).

Construction of BP::β-glucuronidase (BP::GUS) lines

A *BP::GUS* plasmid (pknat1–15, derived from pBI101) containing 5 kb of regulatory sequence upstream of the *BP* start codon (Ori et al., 2000) was a gift from N. Ori and S. Hake. The *GUS* gene and