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The Role of *PHANTASTICA* in Leaf Development

Plant leaves occur in a fantastic variety of sizes and shapes, and the genetic mechanisms behind this variation have long fascinated and challenged plant biologists. Leaves are produced laterally off of the main shoot axis, and may be simple, with a single blade (or lamina) extending from the petiole, or compound, with two or more leaflets per petiole. Leaf primordia emerge from the periphery of the shoot apical meristem (SAM), and leaf blades expand asymmetrically along the proximodistal (base to tip) and mediolateral (midvein to margin) axes. In addition, leaves typically develop distinct polarity along the dorso-ventral axis. The upper (adaxial) surface of a leaf often has a waxier cuticle and fewer stomata than the lower (abaxial) surface. In C3 plants, the mesophyll cells on the adaxial side are arranged into regular columns of palisade parenchyma, whereas the abaxial side consists of more disorganized spongy parenchyma. In addition, most leaf vascular systems have adaxial xylem and abaxial phloem.

Numerous studies, beginning with the work of Ian Sussex and others in the 1950s (Sussex, 1954) have suggested that leaf blade expansion is dependent on the development of abaxial/adaxial polarity. More recently, Waites and Hudson (1995) proposed a model linking abaxial/adaxial polarity to blade expansion based on observations of mutations at the *phantastica* (*phan*) locus in Antirrhinum. *phan* mutations produce a range of leaf morphologies, including radially symmetric leaves that lack adaxial cell types, suggesting that *PHAN* plays a role in development of the adaxial domain. *phan* mutant leaves also produce ectopic blade primordia at novel boundaries between adaxial and abaxial cell types, which led Waites and Hudson (1995) to propose that the juxtaposition of adaxial and abaxial cell types is required for blade outgrowth.

This model is supported by the characterization of *phabulosa* (*phb*) and *phavoluta* (*phv*) mutants in Arabidopsis, the leaves of which lack abaxial cell fates (opposite to the *phan* phenotype), yet likewise develop with radial symmetry and fail to form a blade (McConnell and Barton, 1998; McConnell et al., 2001). These were found to be dominant gain-of-function mutations in genes involved in the specification of adaxial cell fate in wild-type leaves. *PHB* and *PHV* encode homeodomain-leucine zipper (HD-ZIPIII) transcription factors that specify adaxial cell fate, perhaps by restricting the expression of *KANADI* and *YABBY* genes that specify abaxial cell fates (reviewed in Golz and Hudson, 2002). A third HD-ZIPIII protein, REVOLUTA (REV), also acts with PHB and PHV in specifying adaxial cell fate (Otsuga et al., 2001; Emery et al., 2003).

PHAN encodes a MYB domain transcription factor (Waites et al., 1998), and subsequent studies of *PHAN* orthologs *ASYMMETRIC LEAVES1* (*AS1*) in Arabidopsis (Byrne et al., 2000; Ori et al., 2000)

and *ROUGH SHEATH2* in maize (Schneeberger et al., 1998; Timmermans et al., 1999; Tsiantis et al., 1999) demonstrated that a major function of *PHAN* is in the repression of *KNOTTED1*-like homeobox (*KNOX*) genes. *KNOX* genes are important in the maintenance of the SAM, and repression of these genes has been shown to be critical for development of lateral organs (reviewed in Byrne et al., 2001). In Arabidopsis, *AS1/PHAN* negatively regulates *KNOX* genes, including *KNAT1* and *KNAT2*, but is itself negatively regulated by another *KNOX* gene, *SHOOT MERISTEMLESS* (*STM*; Byrne et al., 2002). Recent studies on cell fate determination suggest that *KNOX* genes, such as *STM*, suppress determination signals in the meristem, allowing apical initial cells and their derivatives to proliferate (Veit, 2004).

Kim et al. (2003a and 2003b) proposed that the generation of compound leaves in tomato is regulated by a complex mutually antagonistic relationship in tomato
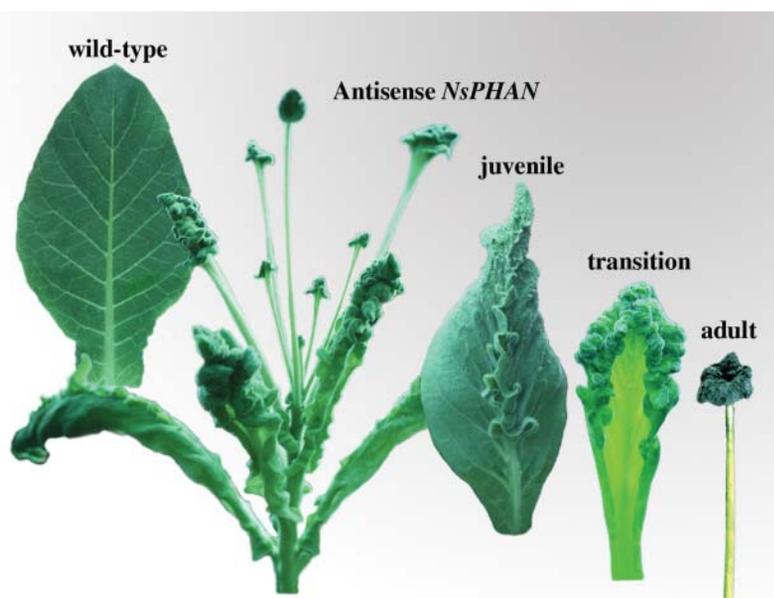
Figure 1. Leaf Phenotypes of Transgenic *N. sylvestris* Expressing an Antisense *NsPHAN* Construct.

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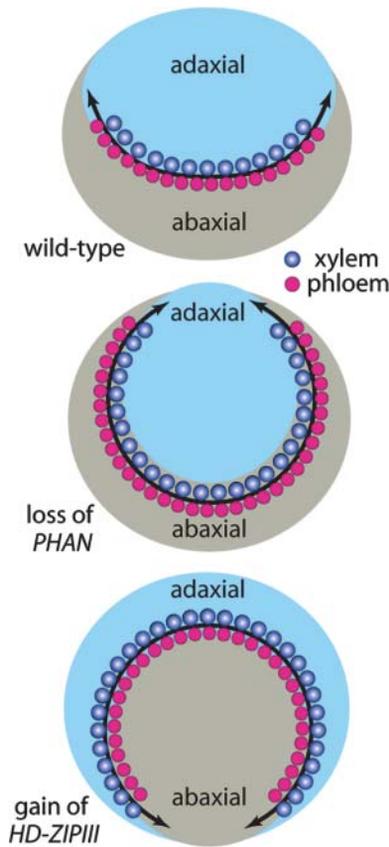
Figure 2. Model Explaining Loss of *PHAN* and Gain of *HD-ZIPIII* Gene Activities in Antirrhinum, Nicotiana, and Arabidopsis.

between *PHAN* and the *KNOX* gene *LeT6* (putative *STM* ortholog in tomato). Compound leaves may be pinnate, with leaflets arranged in succession along the length of the rachis (the central petiole of a compound leaf), or palmate, with a cluster of leaflets radiating from the tip of the rachis. Kim et al. (2003b) have shown that antisense inhibition of *PHAN* expression in tomato reduces the adaxial domain of leaf primordia and transforms pinnate compound leaves into palmate compound leaves. Examination of *PHAN* expression in a variety of species with compound leaves suggested a correlation between *PHAN* expression patterns and the development of compound versus simple leaves.

However, many details of *PHAN* function are unknown, including the precise function

in development of the adaxial domain and the relationship to other genes, such as *PHB*, *PHV*, and *REV*, which specify adaxial cell fate. In this issue of *The Plant Cell*, **McHale and Koning (pages 1251–1262)** describe the Nicotiana ortholog of *PHAN* and investigate *PHAN* function in a series of antisense transgenics in *N. sylvestris*. The authors link distinctly different loss of PHAN phenotypes in juvenile versus adult leaves (Figure 1) to ectopic KNOX expression and present a novel view on the role of *PHAN* in leaf development.

Expression of the *PHAN* ortholog in wild-type *N. sylvestris* (*NsPHAN*) was examined by in situ hybridization. *NsPHAN* mRNA was present throughout P1 and P2 leaf primordia but not in the central zone of the SAM. Later in development, in the P3 and P4 primordia, *NsPHAN* began to show a distinct adaxial pattern of expression, and in expanding leaf blades expression was adjacent to the midvein and lateral veins and in the middle mesophyll where vascular tissue differentiates. Antisense *NsPHAN* transgenic plants were created, which showed no detectable expression of *NsPHAN* mRNA in RNA gel blot expression analysis. Juvenile leaf primordia of antisense plants exhibited normal polarity and initiated leaf blades in the normal position at the adaxial/abaxial boundary but then produced highly disorganized upper mesophyll cells in place of regular palisade mesophyll and ectopic leaf blades along the flanks of major leaf veins on the adaxial surface. The authors propose that ectopic derepression of *KNOX* genes in the absence of *NsPHAN* expression causes the adaxial mesophyll to remain in a partially indeterminate state, in which it retains the capacity for initiation of lateral blade primordia. Thus, in juvenile leaves, it appears that *PHAN* does not specify adaxial cell fate (a function associated with expression of *PHB*, *PHV*, and *REV*) but, through repression of *KNOX* gene expression, regulates adaxial development by promoting the onset of determinacy and differentiation.

KNOX genes have been found to repress the expression of GA20 oxidase genes involved in the biosynthesis of gibberellin (GA) in Arabidopsis (Hay et al., 2002) and

Nicotiana (Tanaka-Ueguchi et al., 1998; Sakamoto et al., 2001). McHale and Koning show that application of exogenous GA to antisense *NsPHAN* plants produced nearly full reversal of the antisense phenotype in juvenile leaves of heterozygous plants. Therefore, at least one function of *PHAN* in developing leaves may be to promote GA biosynthesis, via repression of *KNOX* genes, where the hormone may be required to regulate the organized patterns of cell division and cell expansion in developing adaxial mesophyll tissue.

The adult leaves of antisense *NsPHAN* exhibited a different morphology, characterized by radialization of the petiole and loss of blade formation, as observed in *phan* mutants of Antirrhinum. However, this phenotype did not appear to result from a loss of adaxial cell fate in Nicotiana because the adult petioles showed expression of the adaxial marker *PHV* and maintained the capacity to produce axillary meristems. McHale and Koning propose that, in adult Nicotiana leaves, derepression of *KNOX* genes in the absence of *PHAN* expression causes a distal displacement of radial stem-like patterning from internodes into the leaf petioles. This view is consistent with work in Arabidopsis showing that the *KNOX* gene *KNAT1/BREVIPEDICELLUS* (a homolog of Nicotiana *NTH20*) promotes radialization and elongation of internodes (Douglas et al., 2002; Smith and Hake, 2003).

This may provide a unifying explanation for the radialized petiole phenotypes of Antirrhinum, tomato, and Nicotiana and raises intriguing questions about a functional connection between the KNOX and HD-ZIPIII pathways (Figure 2). Loss of PHAN results in radialization of the leaf midvein (rolled inward toward the shoot, with phloem surrounding xylem) and an apparent constriction of the adaxial domain. This is exactly the opposite of what occurs in the dominant HD-ZIPIII mutants of Arabidopsis, where misexpression of PHB, PHV, or REV causes leaf midveins to roll outward away from the shoot (xylem surrounding phloem) and the adaxial domain is enlarged. Emery et al. (2003) have shown in Arabidopsis that this results directly from loss of microRNA regulation

of REV. Why would ectopic KNOX expression in loss of PHAN mutants produce exactly the opposite result? Does ectopic KNOX expand the domain where miRNAs are targeting HD-ZIPIII transcripts for destruction? Another important question is, are there differences between species or between tissue types (e.g., epidermis, mesophyll, and vascular tissues) in the prominence of the KNOX and HD-ZIPIII pathways in regulating adaxial development? Evidence for a functional link between the KNOX and HD-ZIPIII pathways would be an exciting step forward in our understanding of meristem organization and leaf development.

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