Organ Polarity in Plants Is Specified through the Opposing Activity of Two Distinct Small Regulatory RNAs

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Small RNAs and their targets form complex regulatory networks that control cellular and developmental processes in multicellular organisms. In plants, dorsoventral (adaxial/abaxial) patterning provides a unique example of a developmental process in which early patterning decisions are determined by small RNAs. A gradient of microRNA166 on the abaxial/ventral side of the incipient leaf restricts the expression of adaxial/dorsal determinants. Another class of small RNAs, the *TAS3*-derivated *trans*-acting short-interfering RNAs (ta-siRNAs), are expressed adaxially and repress the activity of abaxial factors. Loss of maize *leafbladeless1* (*lbl1*) function, a key component of the ta-siRNA biogenesis pathway, leads to misexpression of miR166 throughout the initiating leaf, implicating ta-siRNAs in the spatiotemporal regulation of miR166. The spatial restriction of ta-siRNA biogenesis components suggests that this pathway may act non-cell-autonomously in the meristem and possibly contributes to the classic meristem-borne adaxializing Sussex signal. Here, we discuss the key participants in adaxial/abaxial patterning and point out the intriguing possibility that organ polarity in plants is established by the opposing action of specific ta-siRNAs and miRNAs.

Plant shoots are characterized by indeterminate growth. The growing tip of a plant, referred to as the shoot apical meristem (SAM), contains a population of pluripotent stem cells that divide to maintain the SAM and to generate daughter cells from which lateral organs, such as leaves, arise (Fig. 1). Leaves of most seed plants are dorsoventrally flattened and differentiate distinct cell types in the upper and lower leaf surfaces to maximize the capture of sunlight and the exchange of gases that drive photosynthesis. Dorsoventral (adaxial/abaxial) polarity is specified shortly after the emergence of the leaf primordium from the meristem and is thought to reflect inherent positional differences in the developing organ relative to the SAM (Wardlaw 1940). The adaxial, dorsal side of the leaf develops in closer proximity to the tip of the SAM than the abaxial, ventral side (Fig. 1) (for review, see Bowman et al. 2002). Surgical experiments separating the incipient leaf from the remainder of the SAM result in radially symmetric, abaxialized leaves suggesting the existence of a meristem-borne signal that specifies adaxial cell fate (Sussex 1951, 1955). Although the identity of the Sussex signal remains unknown, recent studies implicate small regulatory RNAs in adaxial/abaxial patterning and raise the possibility that a mobile RNA might fulfill the requirements of a positional, polarizing signal. Here, we review the role of microRNAs (miRNAs) and *trans*-acting short interfering RNAs (ta-siRNAs) in leaf polarity and outline recent results suggesting that the opposing activity of two distinct small regulatory RNAs establishes adaxial/abaxial asymmetry in the developing leaf.

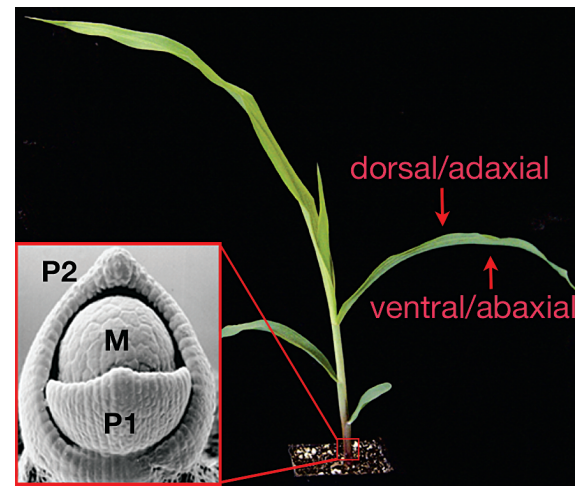

Figure 1. Leaf primordia arise on the flank of the shoot apical meristem (SAM) and establish dorsoventral (adaxial/abaxial) polarity in response to signals from the SAM. Leaves of a 2-week-old maize seedling differentiate distinct dorsal (adaxial) and ventral (abaxial) surfaces. The red box indicates the approximate position of the SAM within the surrounding older leaves. The inset shows a scanning electron micrograph of a maize apex. The population of stem cells at the tip of the meristem (M) permits the reiterative development of the leaf primordia, which emerge from the flank of the SAM. The youngest leaf primordium is indicated as P1, the second youngest as P2, etc.

ESTABLISHMENT OF LEAF POLARITY IN *ARABIDOPSIS*

Several families of putative transcription factors play key roles in establishing adaxial/abaxial polarity in the leaf. Members of the class III family of homeodomain-leucine zipper (HD-ZIPIII) proteins—PHABULOSA (PHB), PHAVOLUTA (PHV), and REVOLUTA (REV)—specify adaxial fate (McConnell et al. 2001; Otsuga et al. 2001; Emery et al. 2003). In contrast, the *KANADI* (*KAN*) genes, which encode transcriptional regulators belonging to the GARP family, act redundantly to

promote abaxial identity (Eshed et al. 2001, 2004; Kerstetter et al. 2001). Although both the *HD-ZIPIII* and *KAN* genes are expressed evenly throughout the incipient leaf (P0), their domains of expression become restricted to the adaxial and abaxial sides of the organ, respectively, shortly after emergence of the primordium from the SAM. Constitutive *KAN* expression leads to the development of a radially symmetric, abaxialized leaf (Eshed et al. 2001, 2004; Kerstetter et al. 2001). Loss of *HD-ZIPIII* function results in a similar phenotype, suggesting that the *HD-ZIPIII* genes act, at least in part, to spatially restrict the *KAN* expression domain (Emery et al. 2003). *HD-ZIPIII* expression is in turn excluded from the abaxial side by the action of the KAN proteins. Thus, the *HD-ZIPIII* and *KAN* genes have a mutually antagonistic relationship, which may reflect their requirement to maintain a stable adaxial/abaxial boundary throughout leaf development.

In *Arabidopsis*, establishment of abaxial identity further requires the activities of members of the *YABBY* and *AUXIN RESPONSE FACTOR* (*ARF*) families (Sawa et al. 1999; Siegfried et al. 1999; Pekker et al. 2005). The *YABBY* genes *FILAMENTOUS FLOWER* (*FIL*) and *YAB3* act, at least in part, downstream of the *HD-ZIPIII* and *KAN* genes, whereas *ARF3/ETT* and *ARF4* affect organ polarity through a distinct pathway (Sawa et al. 1999; Siegfried et al. 1999; Kumaran et al. 2002; Pekker et al. 2005). *ARF3/ETT* is expressed more broadly than *ARF4*, but both transcription factors colocalize in the abaxial domain of leaf primordia, where they act in combination with KAN proteins to promote abaxial fate (Pekker et al. 2005).

Interestingly, both the abaxial determinants *ARF3/ETT* and *ARF4*, as well as the adaxializing *HD-ZIPIII* genes, are targets for RNAi-based regulation (Fig. 2a). *HD-ZIPIII* transcripts contain complementary target sites to the nearly identical microRNAs 165 and 166 (miR165/166), which can direct cleavage of *HD-ZIPIII* mRNAs in vitro (Rhoades et al. 2002; Tang et al. 2003). *ARF3/ETT* and *ARF4* are targets of the recently discovered ta-siRNAs (Allen et al. 2005; Williams et al. 2005a). Thus, in addition to being a key developmental process, the prominent role of small regulatory RNAs in adaxial/abaxial patterning makes leaf polarity an excellent model to dissect the role of small RNAs as developmental signals.

BIOGENESIS AND FUNCTION OF miRNAs

The biogenesis and function of plant miRNAs have been extensively reviewed elsewhere (see Timmermans et al. 2004; Jones-Rhoades et al. 2006; Mallory and Vaucheret 2006). Briefly, miRNAs are processed from

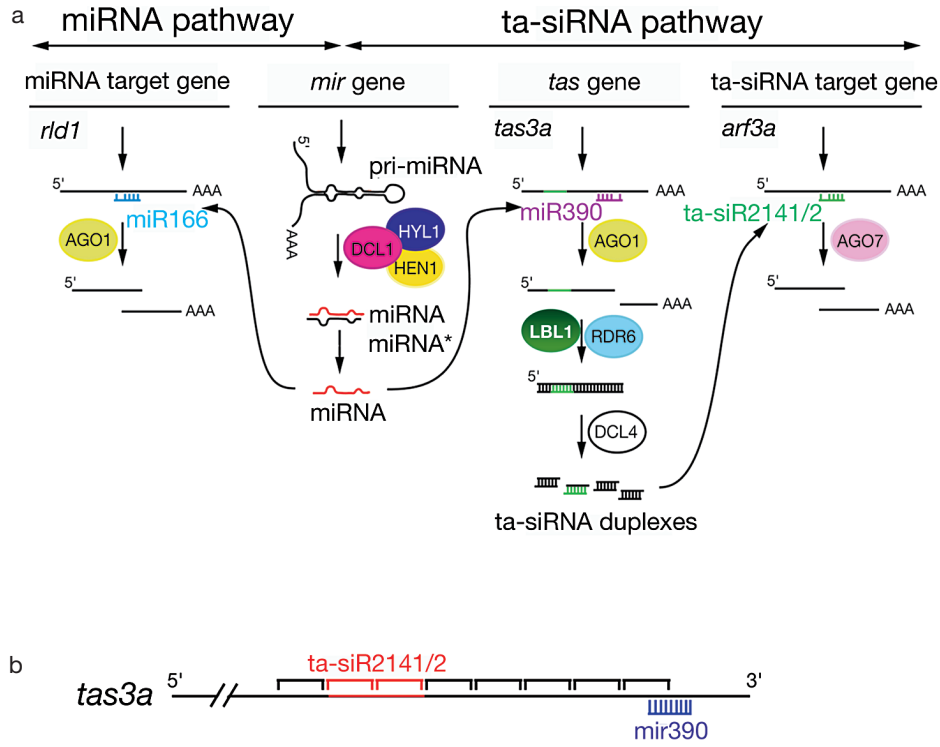

Figure 2. Plant miRNA and ta-siRNA biogenesis and function are interconnected. (*a*) *mir* genes transcribe a primary miRNA transcript (pri-miRNA), which is rapidly processed by DCL1 and HYL1. The resulting imperfect miRNA/miRNA* duplex is 3′-end methylated by HEN1 and loaded onto an AGO1-containing RISC. This complex cleaves target transcripts; *HD-ZIPIII* (*rld1*) transcripts in case of miR166 and *tas3a* mRNAs for miR390. The *tas3a* cleavage fragments are converted into double-stranded RNAs through the activities of LBL1/SGS3 and RDR6, and processed by DCL4 into 21-nucleotide double-stranded siRNAs. The *tas3a*-derived ta-siR2141/2142 acts *in trans* to cleave *arf3a* transcripts via AGO7. (*b*) Diagram of the maize *tas3a* transcript illustrating the 21-nucleotide phased processing of ta-siRNAs, which initiates from the miR390-cleavage site. Black brackets represent putative ta-siRNAs, and red brackets correspond to *Arabidopsis* ta-siR2141/ta-siR2142 siRNA homologs.

RNA polymerase II transcripts that contain a stem-loop structure (Fig. 2a). These transcripts, termed primary miRNAs (pri-miRNAs), are processed by the RNase III enzyme DICER-LIKE1 (DCL1) (Park et al. 2002; Reinhart et al. 2002). Subsequently, the approximately 21-nucleotide mature miRNA becomes incorporated into the RNA-induced silencing complex (RISC), which targets complementary transcripts for site-specific cleavage or translational repression (Chen 2004; Han et al. 2004; Vaucheret et al. 2004; Baumberger and Baulcombe 2005). Plant miRNAs and their targets frequently possess near-perfect complementarity, and this has enabled the identification of many target genes using computational approaches. Interestingly, the known plant miRNAs show a strong propensity to target transcription factors or other genes that regulate critical steps during plant development (Reinhart et al. 2002; Jones-Rhoades et al. 2006). Consequently, mutations in genes associated with miRNA biogenesis or function, such as *ARGONAUTE1* (*AGO1*), *HYPONASTIC LEAVES1* (*HYL1*), and *SERRATE* (*SE*), affect important developmental processes including adaxial/abaxial patterning (Kidner and Martienssen 2004; Grigg et al. 2005; Yu et al. 2005; Yang et al. 2006).

Elucidation of the precise developmental roles of individual miRNAs is, however, complicated by the presence of extensive redundancy. Most *MIR* genes are members of multigene families (see Bartel 2004; Jones-Rhoades et al. 2006). Whereas miRNA families in animals are small and include diverse members, plant miRNA families frequently contain many genes that can produce identical mature miRNA sequences. Family members are likely to have overlapping expression profiles and functions that buffer against the loss of any single miRNA locus, as few loss-of-function alleles of *MIR* genes have been recovered through forward-genetic screens. Dominant gain-of-function miRNA mutants, however, are more common and have revealed developmental roles for several miRNAs. For example, the *meristem enlarged1* and *jabba1-D* mutants both develop fasciated stems and enlarged meristems resulting from altered *MIR166a* and *MIR166g* expression, respectively (Kim et al. 2005; Williams et al. 2005b). Similar gain-of-function mutations for miR156, miR159, miR160, miR164, miR172, and miR319 lead to defects in vegetative and floral organ development, meristem function, and flowering (see Jones-Rhoades et al. 2006).

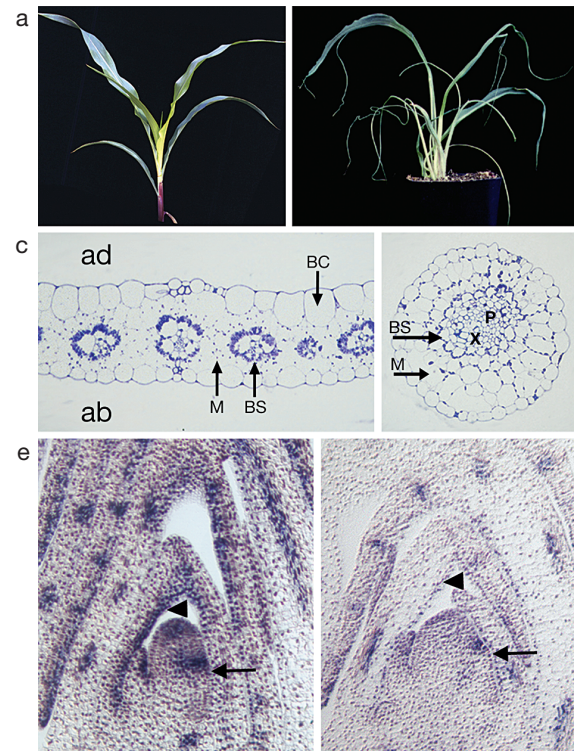

Figure 3. LBL1 is required for the specification of adaxial/dorsal fate. Compared to the wild-type maize seedling (*a*), severe *lbl1* mutants (*b*) develop thread-like, abaxialized leaves. (*c*) Transverse section through a wild-type leaf blade illustrates its dorsoventrally flattened organization, with polar veins surrounded by bundle sheath (BS) and mesophyll cells (M). Bulliform cells (BC) differentiate only in the adaxial (ad) epidermis. (*d*) Severe *lbl1* leaves are radially symmetric and comprise an irregular vascular cylinder surrounded by concentric rings of bundle sheath and mesophyll cells, and abaxial (ab) epidermis. X, xylem; P, phloem. (*e*) Longitudinal section through a wild-type apex showing *rld1* expression in the SAM, vasculature, and on the adaxial side of the incipient (*arrow*) and developing leaf primordia (*arrowhead*). (*f*) In *lbl1*, meristematic and adaxial expression of *rld1* is reduced. (Reprinted, with permission, from Timmermans et al. 1998 and Juarez et al. 2004a.)

ROLE OF MIRNA165/166 IN ADAXIAL/ABAXIAL PATTERNING

As in *Arabidopsis*, adaxial/abaxial asymmetry in maize is established through the polarized expression of *HD-ZIPIII* genes (Juarez et al. 2004b). *rolled leaf1* (*rld1*), which encodes a close homolog of *REV*, is expressed at the tip of the SAM and in a strip of cells from the center of the SAM to the site of leaf initiation (Fig. 3e). In developing leaf primordia, *rld1* is expressed on the adaxial side as well as in the vasculature. The miR165/166 target site in the *HD-ZIPIII* transcripts is conserved between monocots and dicots (Reinhart et al. 2002; Rhoades et al. 2002; Juarez et al. 2004b). Because these lineages last shared a common ancestor more than 100 million years ago, this conservation suggests an important role in plant development for the regulation of *HD-ZIPIII* genes by miR165/166. The significance of this relationship was first demonstrated by the characterization of dominant mutations in the *Arabidopsis HD-ZIPIII* genes (*phb-d*, *phv-d*, and *rev-d*) that abrogate the miR165/166 target site. Such mutations interfere with miRNA-directed transcript cleavage, leading to ectopic, abaxial *HD-ZIPIII* expression and formation of adaxialized leaves (McConnell et al. 2001; Emery et al. 2003; Tang et al. 2003). Disruption of the miRNA165/166 complementary site of maize *rld1* (*Rld1-O*) similarly results in misexpression of *rld1* on the abaxial side of developing primordia and adaxialization of the leaf (Juarez et al. 2004b).

In situ hybridization provides direct evidence that the pattern of miR165/166 expression spatially defines the expression domain of the *HD-ZIPIII* genes (Juarez et al. 2004b; Kidner and Martienssen 2004). miR165 and

miR166 are expressed on the abaxial side of the developing leaf in a pattern complementary to that of the *HD-ZIPIII* genes. Interestingly, in maize, miR166 is most abundant in a group of cells below the incipient leaf, but a gradient of weaker miR166 expression extends into the abaxial side of the newly initiating primordium. In P1 primordia, higher levels of miR166 accumulate abaxially and, in older primordia, miR166 accumulates in a progressively broader domain extending adaxially and laterally (Juarez et al. 2004b). These findings suggest that miR165/166 is a highly conserved polarizing signal that specifies adaxial/abaxial polarity by restricting *HD-ZIPIII* expression to the adaxial side of the leaf in both dicots and monocots (Timmermans et al. 2004). The spatial regulation of *HD-ZIPIII* gene expression by miR165/166 may even predate the origin of angiosperm leaves. The miR165/166-directed cleavage of *HD-ZIPIII* transcripts is conserved in basal lineages of land plants, including bryophytes, lycopods, and ferns (Floyd and Bowman 2004). Organ polarity is a relatively recent landmark in plant development; thus, it is possible that the regulation of *HD-ZIPIII* genes by miR165/166 evolved as a preadaptation that was later co-opted for use in adaxial/abaxial patterning of leaves and other lateral organs (Floyd et al. 2006).

THE TA-SIRNA PATHWAY

Like the miRNAs, ta-siRNAs are processed from long, RNA pol II transcripts that are not predicted to encode for proteins (Fig. 2a). Interestingly, the ta-siRNA precursors (*TAS*) are themselves targets for miRNA-directed cleavage (Allen et al. 2005). However, unlike most miRNA-directed cleavage products, *TAS* cleavage fragments are converted into double-stranded RNAs through the activities of the plant-specific Zn-finger protein SUPPRESSOR OF GENE SILENCING3 (SGS3) and RNA-DEPENDENT RNA POLYMERASE6 (RDR6), and are subsequently processed by DCL4 into 21-bp siRNAs that guide the cleavage of target mRNAs, similar to the action of miRNAs (Peragine et al. 2004; Vazquez et al. 2004; Allen et al. 2005; Gasciolli et al. 2005; Xie et al. 2005; Yoshikawa et al. 2005). Biogenesis of ta-siRNAs thus requires the activity of proteins in the miRNA pathway, such as DCL1 and AGO1, as well as SGS3, RDR6, and DCL4 (Fig. 2a).

Three gene families are known to generate ta-siRNAs in *Arabidopsis*. *TAS1* and *TAS2* are targets of miR173, whereas the production of ta-siRNAs from *TAS3* depends on miR390-mediated cleavage (Allen et al. 2005). Because DCL4 cleavage initiates at the processed end of the *TAS* precursor, ta-siRNAs are generated in a 21-nucleotide phase starting at the miRNA cleavage site (Fig. 2b). Consequently, the ta-siRNAs derived from each *TAS* locus, as well as their potential targets, can be predicted using computational approaches. The *TAS1/TAS2* loci share homology and produce related ta-siRNAs directed against a subset of pentatricopeptide repeat genes and a group of genes of unknown function (Peragine et al. 2004; Vazquez et al. 2004; Allen et al. 2005; Yoshikawa et al. 2005). Mutational analysis indicates that the *TAS1*- and *TAS2*-derived ta-siRNAs have no obvious developmental role (Adenot et al. 2006). In contrast, two of the *TAS3*-derived ta-siRNAs, ta-siR2141/2, regulate the expression of *ARF2*, *ARF3/ETT*, and *ARF4*, which are known to function during shoot morphogenesis and adaxial/abaxial patterning (Allen et al. 2005; Pekker et al. 2005; Williams et al. 2005a).

TAS3 TA-SIRNAS SPECIFY LEAF POLARITY THROUGH REGULATION OF MIR166

Given that the abaxial determinants *ARF3/ETT* and *ARF4* are targets for *TAS3* ta-siRNAs, a role for the ta-siRNA pathway in leaf polarity can be inferred. However, the contribution of this pathway to adaxial/abaxial patterning in *Arabidopsis* is not immediately apparent. *Arabidopsis* mutants that block the biogenesis of ta-siRNAs develop no obvious leaf polarity defects (Peragine et al. 2004; Vazquez et al. 2004; Allen et al. 2005; Adenot et al. 2006). Moreover, the distribution of trichomes on leaves of such mutants, as well on plants expressing a ta-siR2141/2 insensitive allele of *ARF3/ETT*, is inconsistent with the predicted abaxializing phenotype (Peragine et al. 2004; Fahlgren et al. 2006; Hunter et al. 2006).

An essential role for the ta-siRNA pathway in adaxial/abaxial patterning became evident through the cloning and characterization of *leafbladeless1* (*lbl1*) from maize. *lbl1*, in addition to the *HD-ZIPIII* genes, is required for the specification of adaxial fate (Timmermans et al. 1998; Juarez et al. 2004a). Loss-of-function *lbl1* mutations condition an abaxialized leaf phenotype, with the most severely affected *lbl1* leaves becoming radially symmetric and fully abaxialized (Fig. 3a–d) (Timmermans et al. 1998). The recent cloning of *lbl1* showed that it encodes a homolog of the *Arabidopsis* SGS3 protein. LBL1 and the *Arabidopsis* SGS3 protein share 65% amino acid similarity overall, but the degree of sequence similarity is higher in the zinc-finger (92%) and XS domains (79%), which define the SGS3 protein family (Fig. 4) (Bateman 2002).

These findings indicate that SGS3 activity is essential for adaxial/abaxial patterning in maize, and suggest a role for ta-siRNAs in this process. miR173 and the *TAS1/TAS2* loci are not conserved between maize and *Arabidopsis*, but the maize genome includes at least one *mir390* gene and four *tas3* loci (*tas3a–tas3d*) whose transcripts are predicted targets for miR390. Most *tas3*-derived ta-siRNAs are not conserved between maize and *Arabidopsis*, but interestingly, all four maize *tas3* transcripts are predicted to yield copies of ta-siR2141/2 (Fig. 2b). The *tas3* genes, as well as *mir390*, are expressed in vegetative apices, and accordingly, ta-siR2141/2 accumulates in this tissue (F. Nogueira et al., in prep.). ta-siR2141/2 accumulation is severely reduced or abolished in *lbl1* mutants, suggestive of functional conservation between LBL1 and SGS3. As in *Arabidopsis*, of the maize *tas3*-derived ta-siRNAs, only ta-siR2141/2 has clearly identifiable candidate targets, and these include members of the *arf3* gene family (F. Nogueira et al., in prep.). 5′ RACE analysis identified *arf3a* as a direct tar-

Figure 4. *lbl1* encodes a SGS3-like protein. Partial sequencing alignment of LBL1 (DQ832257) and the SGS3 proteins from *Arabidopsis thaliana* (NP_197747.1), *Oryza sativa* (AK064217.1), and *Lycopersicum esculentum* (BT013417.1). The zinc-finger domain (*black*), XS domain (*red*), and coiled coils (*blue*) characteristic of the SGS3 protein family are underlined. Asterisks mark amino acid substitutions identified in severe *lbl1* alleles.

get of ta-siR2141/2, indicating that the ta-siRNA pathway is extensively conserved between maize and *Arabidopsis*. This pathway could be even more ancient, because homologs of miR390 and *TAS3* have been identified in moss, which last shared a common ancestor with the monocots and dicots more than 400 million years ago (Arazi et al. 2005; D. Bartel, pers. comm.). Importantly, *lbl1* is required for the ta-siR2141/2-directed cleavage of *arf3a*, and thus forms an essential component of the ta-siRNA pathway. The observation that LBL1 is essential for adaxial/abaxial patterning in maize thus implies a key role for ta-siRNAs in this process.

lbl1 contributes to leaf polarity by regulating the expression of *HD-ZIPIII* genes (Juarez et al. 2004a; F. Nogueira et al., in prep.). Expression of *rld1* and its paralogs *rld2* and *phb* on the adaxial side of developing leaf primordia is reduced in *lbl1* mutants (Fig. 3e,f). Notably, *HD-ZIPIII* expression at the side of leaf initiation is altered in *lbl1* mutants, suggesting that *lbl1* and the ta-siRNA pathway affect an early step in adaxial/abaxial patterning. In situ hybridization confirms that *lbl1* is most prominently expressed in a dome of cells at the tip of the SAM that extends into the adaxial side of the initiating primordium.

Loss of LBL1 activity leads to misexpression and/or overexpression of ta-siRNA targets and downstream components. Reduced *HD-ZIPIII* expression in *lbl1*, therefore, suggests that one or more antagonists of the *HD-ZIPIII* genes are controlled by the ta-siRNA pathway. Indeed, *lbl1* affects *HD-ZIPIII* expression by regulating the spatiotemporal pattern of miR166 accumulation (F. Nogueira et al., in prep.). miR166 normally accumulates in a graded pattern on the abaxial side of incipient and young leaf primordia. However, in an *lbl1* mutant background, miR166 is ectopically expressed in a torus at the base of the SAM that broadly overlaps with the incipient leaf. Expression of miR166 in the P1 and older leaf primordia also comprises a broader domain, including both adaxial and abaxial sides. Thus, *lbl1* and the ta-siRNA pathway spatially restrict the miR166 expression domain in the SAM and developing leaf primordia, and contribute to organ polarity by setting up the abaxial specific expression gradient of miR166 in the incipient leaf. This presents the intriguing possibility that the opposing activity of two distinct small regulatory RNAs directs the early patterning decision leading to adaxial/abaxial polarity in the incipient leaf; ta-siR2141/2 defines the adaxial side of the leaf by restricting the expression domain of miR166, which in turn delineates the abaxial side by restricting expression of the adaxializing *HD-ZIPIII* genes.

The maize genome includes at least nine *mir166* loci, *mir166a* through *mir166i*. All *mir166* genes are expressed within the vegetative apex, but the spatiotemporal expression profiles vary among family members (F. Nogueira et al., unpubl.). The ta-siRNA pathway affects the accumulation of just a subset of *mir166* precursors. Transcript levels for *mir166c* and *mir166i* are increased in the meri-stem of *lbl1* as compared to wild type, whereas *mir166a* precursor levels are reduced in the mutant (F. Nogueira et al., in prep.). This suggests that the loss of ta-siRNA- directed repression of *mir166c* and *mir166i* underlies, at least partially, the ectopic miR166 accumulation in *lbl1* incipient primordia and resulting abaxialization of *lbl1* leaves.

How might the ta-siRNA pathway restrict miR166 expression? Despite our increasing understanding of miRNA biogenesis and function, little is known about the regulation of *MIR* genes themselves. ARF proteins are transcription factors that mediate auxin-dependent gene regulation through binding to specific sequence motifs within promoters of auxin-regulated genes (Ulmasov et al. 1999). Although it will require gain- or loss-of-function *arf3* mutants to assess the contribution of these transcription factors to the spatiotemporal regulation of miR166, it is not dif-

ficult to envision that the ta-siRNA pathway may control the expression of specific *mir166* family members via *arf3* genes. Additionally, miRNA accumulation is likely regulated at the posttranscriptional level (Bollman et al. 2003; Yang et al. 2006). The primary *mir166i* transcript includes a sequence motif with modest complementarity to ta-siR2141/2. Given that the ta-siRNA pathway is expected to occur in the nucleus (Vaucheret 2006), this observation suggests that the spatiotemporal pattern of miR166 expression may be regulated directly by this ta-siRNA. This possibility is particularly intriguing because it presents a scenario in which miRNA precursors are themselves controlled by small RNAs. Moreover, leaf polarity would be specified through a cascade of direct small regulatory RNA interactions in which miR390-directed cleavage of *TAS3* triggers the production of ta-siR2141/2, which in turn processes *mir166i* required for the polarized expression of miR166 and adaxial/abaxial patterning of the newly initiated leaf.

DIVERSE CONTRIBUTIONS OF THE TA-SIRNA PATHWAY TO LEAF POLARITY IN DISTINCT PLANT LINEAGES

Despite uncertainty regarding the precise mechanism by which the ta-siRNA pathway regulates miR166 expression, the defects observed in *lbl1* clearly demonstrate a critical role for ta-siRNAs in adaxial/abaxial patterning by establishing the abaxial expression gradient of miR166 in the incipient leaf. These findings also highlight important differences between *Arabidopsis* and maize; namely, even though the ta-siRNA pathway is extensively conserved between these species, *Arabidopsis* mutants that block ta-siRNA biogenesis display no obvious leaf polarity defects (Peragine et al. 2004; Vazquez et al. 2004; Allen et al. 2005). The differential reliance on the ta-siRNA pathway for adaxial/abaxial patterning in part reflects redundancy of the pathway in *Arabidopsis* with *ASYMMETRIC LEAVES1 (AS1)* and *AS2*. Double mutants affecting the ta-siRNA pathway as well as *AS1* or *AS2* function develop weakly abaxialized leaves (Li et al. 2005; Garcia et al. 2006; Xu et al. 2006).

In addition, divergence in the nature or function of downstream targets, or the time during leaf development at which downstream targets act, could greatly influence the contribution of the ta-siRNA pathway to adaxial/abaxial patterning in different plant species. For instance, whereas the *Arabidopsis* ta-siRNA pathway represses *FIL* expression (Li et al. 2005; Garcia et al. 2006; Xu et al. 2006), members of the maize *yabby* gene family closely related to *FIL* are positively regulated by *lbl1* (Juarez et al. 2004a). Additionally, although expression analysis suggests that ta-siRNAs contribute to the regulation of miR165/166 in *Arabidopsis* (Li et al. 2005; Xu et al. 2006), disruption of the ta-siRNA pathway affects expression of specific *MIR165/166* family members only during later stages of leaf development (F. Nogueira et al., unpubl.). Distinctly, the maize ta-siRNA pathway acts foremost in the SAM to regulate miR166 expression in the newly initiated primordium, and thus directs the early decisions in adaxial/abaxial patterning (F. Nogueira et al., in prep.).

SMALL RNAs AS POTENTIAL MOBILE SIGNALS IN PLANTS

The antagonistic interaction between ta-siRNAs and miR166 highlights the complexity that can be found in small RNA regulated pathways, and illustrates the important role of small RNAs in pattern formation during development. This unique interaction also raises the question whether small RNAs contribute to the production or perception of positional information from the SAM required for adaxial/abaxial patterning. Due to their intrinsic high specificity, the idea of mobile—possibly morphogenic—small RNA signals in plants is tantalizing. However, evidence so far suggests that miRNAs act largely cell-autonomously (Parizotto et al. 2004; Alvarez et al. 2006). siRNAs generated during posttranscriptional or virus-induced gene silencing, on the other hand, are mobile, and their movement is the basis for the non-cell-autonomous nature of these silencing processes (Himber et al. 2003; Voinnet 2005). Interestingly, cell-to-cell movement is restricted to 21-bp DCL4-dependent siRNAs, whereas the 24-bp siRNAs generated by DCL3 act strictly cell-autonomously outside the phloem (Dunoyer et al. 2005). This suggests that, if produced by the correct mechanism or channeled into the correct pathway, small RNAs can traffic between cells.

In this regard, the involvement of ta-siRNAs in the specification of adaxial fate is particularly intriguing. Their biogenesis requires DCL4, presenting the possibility that this novel class of siRNAs may be able to move from cell to cell. Indeed, ectopic expression of miR166 in the *lbl1* mutant also occurs outside the normal *lbl1* expression domain. This nonoverlapping expression pattern, along with a role for ta-siRNAs in establishing a gradient of miR166 on the abaxial side of the incipient leaf, suggests that the ta-siRNA pathway may operate non-cell-autonomously in the SAM (F. Nogueira et al., in prep.). ta-siRNAs thus constitute a plausible component of the Sussex signal. Consistent with this notion, the most severely affected *lbl1* mutant leaves resemble the abaxialized leaves that arise following the surgical separation of leaf initials from the tip of the SAM (Fig. 3d) (Sussex 1951; Timmermans et al. 1998).

CONCLUSIONS

The finding that *lbl1* encodes a key component of the ta-siRNA pathway reveals an essential role for ta-siRNAs in adaxial/abaxial patterning. In addition, leaf polarity requires the activity of miR165/166. The importance of small regulatory RNAs in adaxial/abaxial polarity may reflect the need to rapidly change transcription profiles that underlie cell fate changes, or to maintain a precise balance between adaxial and abaxial determinants during primordium growth, but could also reflect a role of small RNAs acting as positional cues. The expanded uniform expression of miR166 in incipient primordia of *lbl1* indicates that ta-siRNAs promote adaxial fate by restricting miR166 expression to the abaxial side of the initiating leaf. Organ polarity in

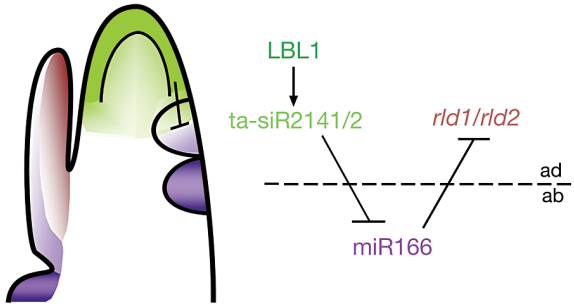

Figure 5. Organ polarity may be specified through the opposing activity of two distinct small regulatory RNAs. *lbl1* (*solid green*) is expressed in a dome of cells at the tip of the SAM, suggesting the site of *TAS3* ta-siRNA biogenesis. miR166 (*purple*) accumulates most prominently below the incipient leaf, but also in a graded pattern on the abaxial side of this primordium. ta-siR2141/2 (*pale green*) may move from its side of synthesis into the adaxial side of the initiating leaf. ta-siR2141/2 locally restricts miR166 expression, which in turn represses expression of the adaxial determinants *rld1* and *rld2* (*red*). The opposing activities of ta-siR2141/2 and miR166 thus set up polarity in the developing leaves.

plants may thus be specified through the opposing activities of ta-siR2141/2 and miR166 (Fig. 5).

The possibility that ta-siRNAs can act as mobile signals, perhaps because of their specific association with DCL4, suggests a mechanism by which ta-siR2141/2 establishes organ polarity. The local biogenesis of ta-siR2141/2 in the SAM followed by its movement through the adjacent tissue would result in a concentration gradient across the initiating leaf, which sets up a complementary gradient of miR166. A defined balance between ta-siR2141/2 and miR166 activities may specify leaf polarity. Such a balance may result through regulated accumulation of each small RNA or through their relative efficacies of cleaving target transcripts. The number of cells over which a siRNA can move is correlated directly with its abundance, suggesting a dilution of the siRNA signal away from its point source (Himber et al. 2003; Voinnet 2005). The relatively low abundance of ta-siR2141/2 (Allen et al. 2005; Williams et al. 2005a) or the involvement of an AGO7-based RISC in the *TAS3* ta-siRNA pathway could therefore be extremely significant for proper adaxial/abaxial patterning.

Although the concept of the Sussex signal has existed for more than 50 years, only recently have we started to better understand the complex genetic network that specifies adaxial/abaxial polarity. The recognition of small RNAs as early regulators of lateral organ polarity implicates them as potential polarizing signals that could perhaps contribute to the Sussex signal.

ACKNOWLEDGMENTS

The authors thank Shahinez Madi, Krista Marran, and Amanda Grieco for excellent technical assistance. This research was supported by grants from the U.S. Department of Agriculture (06-03420) and the National Science Foundation (0615752) to M.T.; F.N. was funded in part by a fellowship from the Cold Spring Harbor Laboratory Association; and D.C. is a National Science Foundation graduate research fellow and a George A. and Marjorie H. Matheson fellow.

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