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Leaf polarity establishment and the two sets of regulators

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ABSTRACT

Proper leaf morphogenesis is evolutionarily important for higher plants in enabling them the maximum photosynthesis capacity. This complex developmental process comprises of several stages. The establishment of leaf polarity along the adaxial (doral)-abaxial (ventral) axis occurs in young leaf primordia and is critically required for subsequent leaf blade expansion. This polar patterning results in distinct anatomical and morphological structures between the upper and the lower sides of the leaf. The first recognition of leaf polarity began with a classical microsurgical study on potato shoot tips over 50 years ago. Subsequently, important leaf polarity regulators have been identified through genetic and molecular studies. This review is mainly focused on characterization of Arabidopsis key determinants that regulate leaf polarity establishment at various levels including transcriptional, post-transcriptional, translational and post-translational. Recent developments in leaf polarity study are also provided.

Keywords: adaxial, abaxial, leaf polarity, leaf development, Arabidopsis

INTRODUCTION

Plant photosynthesis depends largely on correct leaf development. Leaves are initially formed as leaf primordia flanking the shoot apical meristem. During the early stages, a developmental patterning is established along the adaxial-abaxial axis. The outcomes of this developmental process are morphological and anatomical differences between the adaxial and abaxial sides of the leaf (Fig. 1). Trichomes are more abundant on the adaxial side compared to the abaxial side (Fig. 1a). Leaves generally appear dark green and light green on the adaxial and abaxial surface, respectively. This is because of the anatomical distinction between the two sides. The adaxial subepidermal mesophyll layer is mainly composed of tightly packed, regular-shaped palisade cells (Fig. 1b). This unique characteristic of the adaxial mesophyll layer maximizes its ability in light harvesting. In contrast, the abaxial mesophyll layer mainly consists of loosely packed, irregular-shaped spongy cells that are associated with large

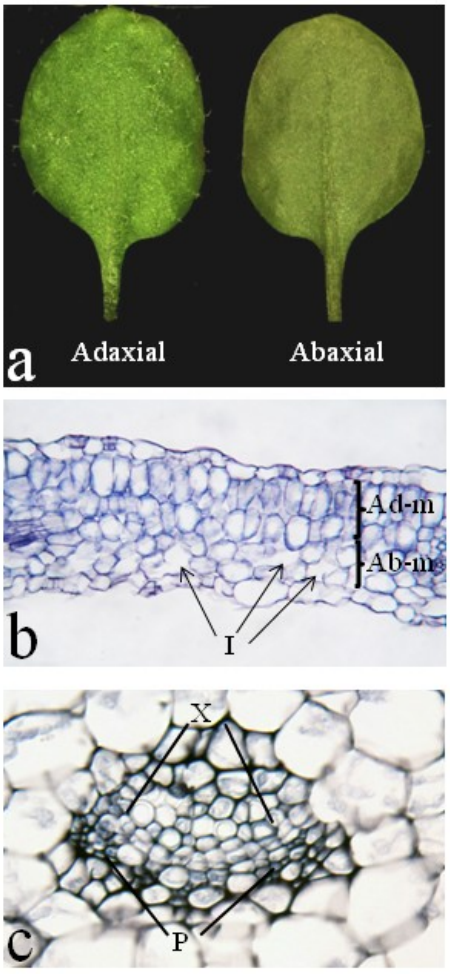

Figure 1 Leaf polarity is established along the adaxial-abaxial axis. (a) Trichomes are generally more abundant on the adaxial side than the abaxial side. (b) The adaxial mesophyll layer is anatomically different from the abaxial mesophyll layer. (c) Xylem is positioned on the adaxial side while phloem is located on the abaxial side. (Ad-m = adaxial mesophyll layer, Ab-m = abaxial mesophyll, I = intercellular spaces, X = xylem, P = phloem). See color figure on the journal website.

intercellular spaces facilitating gas exchange (Fig. 1b). Xylem, a water and mineral transporting tissue, is located towards the adaxial side whereas phloem, a sugar conducting tissue, is positioned towards the abaxial side (Fig. 1c). As leaves develop, leaf polarity is further propagated and maintained throughout the leaf suggesting the presence of two sets of regulatory determinants for adaxial and abaxial cell fate.

First recognition of leaf polarity establishment originated from a microsurgical study on potato shoot tips in 1955 (Sussex, 1995). Separation of young leaf primordia from the shoot apical meristem results in the formation of radially symmetric organs or radialized leaves that resemble a needle and lack leaf blades. Anatomical analysis of these misshaped lateral organs revealed that the vascular bundle is composed of phloem surrounding xylem indicating the misregulated abaxialization. Additionally, the absence of leaf blade suggests that proper leaf polarity establishment is required for leaf blade expansion (Sussex, 1955). Thus, it was hypothesized that meristem generates molecules required for adaxialization (Sussex, 1955). Fifty years later, the microsurgical experiment was repeated and further elaborated by a laser ablation analysis on tomato shoot tips (Reinhardt *et al.*, 2005). Tangential separation of tomato leaf primordia from the shoot apical meristem causes leaf polarity defects similar to what was observed in potato. Additionally, partial removal of the meritematic epidermal (L1) layer that is connected to leaf primodia also results in

similar defects. These results confirm the existence of the hypothetical molecules signifying adaxial cell fate on the meristem-connected side of a leaf primordium while the other side of the primordium may inherently acquires abaxial cell fate (Reinhardt *et al.*, 2005). Although the identity of this adaxialization signaling molecule is still unknown, several regulatory genes for leaf polarity establishment have been described. A number of early studies have demonstrated that leaf polarity determinants form a regulatory network that operates at the transcriptional level (Fig. 2). However, subsequent studies have suggested additional players at other regulatory stages including post-transcriptional, translational and post-translational (Fig. 2).

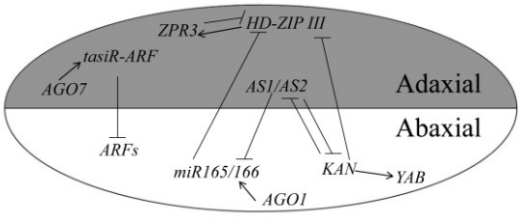

Figure 2 A tentative regulatory network that regulates the establishment of leaf polarity. Adaxialization and abaxialization promoting genes are placed on their corresponding domain.

Transcriptional regulation of leaf polarity

The majority of regulatory genes promoting leaf polarity that have been discovered so far are transcription factors. These proteins have been proposed to function through antagonistic interactions such that expression of abaxial determinants in the abaxial domain suppresses expression of adaxial regulators and vice versa (Fig. 2). The first insight into this complex regulatory network is derived from the study of an *Antirrhinum majus* mutant called *phantastica* (*phan*). Single recessive mutations in the *PHAN* gene cause various degrees in leaf morphological defects (Waites and Hudson, 1995). Cotyledons and the first three pairs of leaves are wider and adopt the heart shape as opposed to narrower, elliptic wild-type leaves. Some leaves produced at later stages only display the loss of leaf polarity at the proximal portion while some are produced as a completely-radialized leaves exhibiting abaxial features. This indicates *PHAN* functions in the determination of adaxial cell fate in leaf primordia. A molecular study has demonstrated that *PHAN* encodes a transciption factor containing Myb domains and is expressed uniformly in leaf primordia to suppress expression of class I *KNOX* genes that promote shoot apical meristem formation (Waites *et al.*, 1998). Thus, its function in adaxial determination may depend upon additional regulators (Waites *et al.*, 1998). The discovery of *PHAN* involvement in leaf polarity has led researchers to extensively explore other regulators in the model plant Arabidopsis.

Owing to the availability of the whole-genome information and a large collection of mutant accessions, several Arabidopsis genes regulating leaf polarity establishment have been identified. A protein family of class III homeodomain-leucin zipper (HD-ZIP III)

transcription factors has been proposed to function in adaxial signaling and the initiation of the shoot apical meristem (Emery *et al.*, 2003). These proteins include *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), *REVOLUTA* (*REV*), and *CORONA* (*CNA*) (Emery *et al.*, 2003). Single, recessive mutations in any of these genes do not result in severe leaf defects, due to their genetic redundancy. For example, *phv* and *phb* single, recessive mutants are aphenotypic whereas the *rev* single, recessive mutant (Fig. 3b) only shows defects in floral meristems (Talbert *et al.*, 1995; Baima *et al.*, 2001; Emery *et al.*, 2003; Prigge *et al.*, 2005). However, the dramatic loss of leaf polarity establishment has been demonstrated in gain-of-function and combinatorial mutants. The gain-of-function *phb-1d* mutant only forms radialized organs displaying adaxial features as well as ectopic formation of shoot apical meristems on the lower portion of the organs (McConnell and Barton, 1998; McConnell *et al.*, 2001). *Phb phv rev* and *phb rev cna* triple mutants produce radialized apical structures with abaxial features and fail to form the shoot apical meristem (Emery *et al.*, 2003; Prigge *et al.*, 2005). The observed phenotypes of these mutants further confirm the intimate link between the shoot apical meristem and adaxialization. Consistent with their functions, expression of *HD-ZIP III* genes is restricted to the shoot apical meristem and the adaxial domain. Along with HD-ZIP III, ASYMMETRIC LEAVES (AS) 1 and AS2 are additional transcription factors that specify adaxial cell identity in Arabidopsis. AS1 is a Myb-domain containing protein, and a sequence analysis suggests that it is a homolog of PHAN (Byrne *et al.*, 2000). In contrast to *phan* mutants, *as1* single, recessive mutants only exhibit triangular-shaped, lobed, asymmetric leaves with downward-curling edges and a wavy surface (Fig. 3c) (Byrne *et al.*, 2000). AS2 is a protein member of the LATERAL ORGAN BOUNDARIES (LOB) family. Leaf phenotypes of *as2* are relatively similar to those of *as1*, except a unique formation of leaflet-like structures that are present on the petioles in *as2* mutants (Fig. 3d) (Lin *et al.*, 2003). Despite the resemblance between their mutant phenotypes, expression patterns of *AS1* and *AS2* are not similar. *AS1* is expressed throughout leaf primordia while *AS2* expression is exclusive to the adaxial domain. Over-expression of *AS1* and *AS2* also results in distinct phenotypes. While *AS1*-overexpressing plants only display a reduced-stature phenotype, *AS2*- overexpressing plants produce

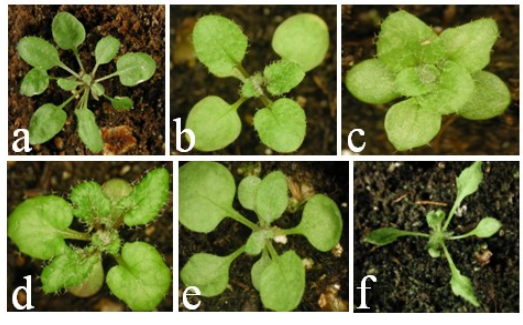

Figure 3 Arabidopsis Enkheim-2 wild type (a) and examples of leaf polarity mutants including *rev1-1* (b), *as1-1* (c), *as2-1* (d), *kan1-1* (e) and *kan1 kan2* (f).

narrow leaves with adaxial identity on the abaxial side (Xu *et al.*, 2003). This suggests different impacts of AS1 and AS2 in adaxialization. However, AS1 and AS2 may exert their functions through a common developmental pathway as indicated by a protein study that demonstrates their direct protein-protein interaction (Xu *et al.*, 2003).

KANADI (KAN) is a protein family that contains Myb-like GARP DNA-binding domain and is responsible for promoting abaxial cell identity on the abaxial side of the leaf (Husbands *et al.*, 2009). Genetic studies show that there is a genetic redundancy between members of this protein family. The first leaf pair produced by *kan1* mutants (Fig. 3e) curls upward and adopts a cup shape (Kerstetter *et al.*, 2001). Spongy mesophyll cells become less irregular-shaped and are associated with smaller intercellular spaces compared to wild type (Kerstetter *et al.*, 2001). *Kan2* leaves are indistinguishable from wild type leaves (Eshed *et al.*, 2001). However, dramatic defects in leaf morphology have been observed in combinatorial mutants. *kan1 kan2* double mutants (Fig. 3f) produce narrow leaves with pointed tips and ectopic abaxial outgrowth (Eshed *et al.*, 2004). *kan1 kan2 kan3* triple mutants form radialized leaves that indicate the loss of leaf polarity establishment (Eshed *et al.*, 2004). In contrast, over-expression of *KAN1* results in the loss of the shoot apical meristem, the absence of vascular bundles in cotyledons and the abaxial identity in the internal tissue of cotyledons (Kerstetter *et al.*, 2001). This line of evidence suggests the role of *KAN* genes in leaf abaxialization.

The molecular function of *KAN1* in leaf polarity has been demonstrated by gene expression and mutant analyses. The loss of *KAN* genes in *kan1 kan2 kan3* triple mutants results in expression of *PHB* in both adaxial and abaxial domains (Eshed *et al.*, 2004). Similarly, expression of *PHV* and *REV* is restricted in the adaxial domain in wild type but expands into the abaxial domain in *kan1 kan2* double mutants (Eshed *et al.*, 2001). These observations suggest a possible role of KAN as suppressors for expression of adaxial determinants in the abaxial domain (Fig. 2). This is strongly supported by a study on a dominant mutant allele *as2-5D*, displaying cupped, adaxialized leaves similar to those of *kan1* single mutants (Wu *et al.*, 2008). Upon characterization of the mutation in *as2-5D,* the study shows that a mutation in an upstream element in the *AS2* promoter causes the mutant phenotypes and uniform *AS2* expression in leaf primordia. This is due to a reduction of its binding affinity to KAN1 that transcriptionally suppresses *AS2* expression in the abaxial domain. Additionally, *AS2* may function as a suppressor of *KAN1* as well. Expression of *KAN1* expands throughout leaf primordia of *as2* loss-of-function mutants and is found only on the base of leaf primordia in *as2-5D.* Taken together, these findings suggest an antagonistic interaction between *KAN1* and *AS2* as part of the regulatory network that controls leaf polarity (Fig. 2).

AUXIN RESPONSE FACTOR (*ARF*) genes *ARF3* and *ARF4* encode transcription factors that mediate plant responses toward the phytohormone auxin (Tiwari *et al.*, 2003). Leaves of *arf3* and *arf4* single mutants are relatively similar to those of wild type (Pekker *et al.*, 2005). The implication of the *ARF* genes in leaf abaxialization is derived from a phenotypic analysis of various double and triple mutants (Pekker *et al.*, 2005). The radialized leaf phenotype caused by *KAN2* over-expression is partially rescued by the mutation in *ARF3.* Leaf morphology of *kan1 kan2* double mutants is similar to that of *arf3 arf4* double and *kan1 kan2 arf3* triple mutants. These genetic interactions suggest an overlap between the functions of *ARF* and *KAN* in leaf development. Despite the epistatic interaction of mutations in *KAN* over *ARF,* a gene expression analysis has shown that *ARF3* and *ARF4* are unlikely direct downstream targets of KAN transcription factors because expression patterns of *ARF3* and *ARF4* are not altered in leaf primordia of *kan1 kan2* double mutants (Pekker *et al.*, 2005). This indicates that *KAN* and *ARF* may interact with different targets and contribute to leaf abaxialization in the same pathway. The involvement of *ARF* genes also suggests the importance of auxin in leaf development.

FILAMENTOUS FLOWER (FIL) and *YABBY (YAB) 3* are members of the *YAB* gene family that promote abaxialization (Siegfried *et al.*, 1999). Similar to other families of leaf polarity determinants, a genetic redundancy between members of the *YAB* gene family has been observed (Siegfried *et al.*, 1999). The phenotypes of *fil* and *yab3* single mutant plants are indistinguishable from those of wild type. In contrast, leaf polarity defects are present in *fil yab3* double mutants (Siegfried *et al.*, 1999). *Fil yab3* double mutant leaves display abaxial epidermal cells whose characteristics resemble those on the adaxial domain, indicating the loss of abaxial cell identity. Transgenic plants overexpressing *YAB* genes display abaxialized leaves and the absence of the shoot apical meristem. The functions of *YAB* genes in abaxialization is linked to those of *KAN* genes based on a gene expression study and mutant analysis (Eshed *et al.*, 2004). Expression of *YAB3* is detectable on the abaxial domain in the wild type background but disappears from leaf primodia of *kan1 kan2 kan3* triple mutant plants. Ectopic expression of *KAN2* results in uniform expression of *FIL* in leaf primordia. In addition, mutations in *YAB* genes enhance leaf morphological and polarity defects of *kan1 kan2* double mutants. The first two leaves of *kan1 kan2 fil yab3* quadruple mutants appear radialized, and cells of the mesophyll layers of subsequent leaves are uniformly adaxialized. Taken together, proper expression of *YAB* requires abaxial signals from *KAN*, and, they together establish the abaxial domain (Eshed *et al.*, 2004). Another study suggests that YAB proteins may form homodimer or heterodimers (YABBYs) and the transcriptional corepressors leunig and leunig.

Post-transcriptional regulation of leaf polarity

While the aforementioned regulators are transcription factors, several lines of evidence have shown that other leaf-polarity determinants exert their functions at different levels ranging from post-transcriptional to post-translational. They also interact with the transcriptional regulators to promote leaf patterning along the adaxial-abaxial axis (Fig. 2). The transcriptional exclusion of *HD-ZIP III* gene expression in the abaxial domain has been primarily linked to the suppressing activity of abaxial determinants *KAN* (Eshed *et al.*, 2001). However, characterization of dominant alleles *phb-1d*, *phv-1d* and *rev-10d* mutants has revealed additional post-transcriptional regulation of the *HD-ZIP III* genes (McConnell *et al.*, 2001; Emery *et al.*, 2003). Mutations in these dominant allelic mutants are located around the area that encodes a START consensus domain conserved among HD-ZIP III members, prompting a speculation on the role of this domain in HD-ZIP III functions. However, further studies have proved otherwise. The nucleotide sequence of this area is the target site of microRNAs (miRNAs) from the *miR165/166* group that is expressed in the abaxial domain and the shoot apical meristem (McConnell *et al.*, 2001; Emery *et al.*, 2003). Transcripts produced by *phb, phv* and *rev* dominant alleles are resistant to RNA cleavage mediated by *miR165/166*, proving their function in leaf polarity by directing *HD-ZIP III* transcripts for post-transcriptional RNA degradation in the abaxial domain. Additionally, over-expression of *miR166* down-regulates the expression levels of *PHB* and *REV* in lateral organs (Williams *et al.*, 2005). Another genetic study has shown that *AS1* and *AS2* are likely involved in suppression of *miR165/166*, and *as1* and *as2* mutant phenotypes may be partially caused by changes in expression of *HD-ZIP III* gene (Li *et al.*, 2005). Consistently, a mutation in *ARGONAUTE* (*AGO*) *1*, encoding a protein associated with miRNA-mediated gene silencing, results in radialized organs with adaxial identity (Kidner and Martienssen, 2004; Vaucheret *et al.*, 2004). On the other hand, another group of small RNA molecules called *trans-acting* short-interfering RNA (tasiRNA)-*ARF* has been shown to target *ARF3* and *ARF4* transcripts for cleavage suggesting its involvement in adaxialization in leaf primordia (Allen *et al.*, 2005). Mutations in *AGO7*, required for the generation and/or stability of *tasiR-ARF*, also result in the up-regulation of *ARF3* and *ARF4* (Hunter *et al.*, 2006). These lines of evidence indicate the important roles of small RNA molecules and post-transcriptional regulation in specifying leaf adaxial-abaxial polarity.

Possible roles of translational machineries in leaf polarity establishment

At the translational level, two studies have recently demonstrated the contribution of ribosomal proteins toward leaf polarity establishment (Pinon *et al.*, 2008; Yao *et al.*, 2008). Ribosomes are composed of several components including various protein subunits

and rRNA (Bailey-Serres, 1998). Although only one copy of each ribosomal protein is incorporated into the ribosome, plant ribosomal proteins are present in multi-gene families (Barakat *et al.*, 2001). Each paralog of plant ribosomal protein families is actively expressed but may accumulate at different levels. Previous studies from two different research groups have shown that *piggyback (pgy) 1* and *asymmetric leaves1/2 enhancer (ae) 5* mutants, whose the ribosomal protein genes *RPL10* and *RPL28A* are disrupted, commonly exhibit a pointed leaf phenotype (Pinon *et al.*, 2008; Yao *et al.*, 2008). These single mutants also similarly enhance leaf polarity defects in the *as1, as2* and *rev* single mutant backgrounds (Pinon *et al.*, 2008; Yao *et al.*, 2008). However, additive effects are observed only in *pgy1 kan1 kan2* triple mutants whereas a synergistic interaction similar to the *kan1 kan2 kan3* triple mutant is found in *ae5 kan1 kan2* triple mutants (Pinon *et al.*, 2008; Yao *et al.*, 2008). These results suggest the distinctive degrees of contributions between different ribosomal proteins regarding leaf polarity.

Post-translational regulation of HD-ZIP III proteins

LITTLE ZIPPER (ZPR) post-translationally regulates establishment of leaf polarity. *ZPR* genes encode small proteins containing a leucine-zipper domain, which is similar to that specifically found on the HD- ZIP III transcription factors (Wenkel *et al.*, 2007). Based on an *in vitro* protein-protein interaction and a yeast-two-hybrid assay, ZPR is proposed to bind to HD-ZIP III transcription factors through this domain (Wenkel *et al.*, 2007; Kim *et al.*, 2008). This protein-protein interaction in turn prevents the dimerization of HD-ZIP III transcription factors that is required for their DNA binding activities and adaxialization signaling. Consistently, over-expression of *ZPR3* results in the formation of radialized organs that display abaxial features (Wenkel *et al.*, 2007). Remarkably, the expression level of *ZPR3* is elevated by ectopic expression of *REV,* and its adaxially expression pattern also coincides with *HD-ZIP III*. This suggests the potential feedback regulation between *ZPR* and *HD-ZIP III* (Wenkel *et al.*, 2007) (Fig. 2).

Recent developments and future prospects in the study of leaf polarity

The process in establishing polar patterning in leaf primordia occurs early and is critically important for proper leaf morphogenesis. It involves a number of players that operate at a broad spectrum of gene expression. Identification of the molecular functions of and the interactions between the regulators has led us to construct a tentative regulatory network. Over the past few years, much progress has been made in the field of leaf polarity study. This includes the characterization of additional regulators, newly discovered interactions between regulators, the identification of direct target genes of the major transcriptional regulator. Following are examples of recent developments. Two

WUSCHEL-RELATED HOMEOBOX (*WOX*) genes, including *WOX1* and *PRS/WOX3*, have been proposed to represent a new set of regulators described as middle domain-specific regulators (Nakata *et al.,* 2012). This is due mainly to their expression patterns in the central domain of leaf primordia situated between the adaxial and the abaxial domains. Loss of function of these genes in combination with either *as2* or *fil yab* mutant backgrounds compromises leaf polarity and causes the formation of radialized organs. Additionally, KAN is able to repress *WOX1* and *WOX3* in the abaxial domain, suggesting their functions downstream of KAN in the leaf polarity pathway.

The mutation on the *AE7* gene has been found to enhance leaf polarity defects of the *as2* mutant (Yuan *et al.,* 2010). *AE7* encodes a protein of 157 amino acids that belongs to the domain of unknown function 59 protein superfamily. Although the molecular function of AE7 is not known, phenotypic and molecular analyses of the *ae7* mutant reveal the defects in cell proliferation specifically at the G2-M transition stage. This is because of the increased levels of the *CDCB1;1* and *CDKB1;1*, G2-M phase specific markers. This has led to the investigation of the cell cycle defects in *ae3* and *ae5* where the genes encoding 26 proteasome and the ribosomal protein *RPL28A* are mutated. Interestingly, *ae3* and *ae5* exhibit the cell cycle defects similar to those of *ae7.* Taken together, the phenotypes of the *ae* mutants suggest that cell division is potentially one of the mechanisms contributing to the establishment of leaf polarity. A transcriptional repressor complex consisting of LEUNIG (LEU), LEUNIG HOMOLOG (LUH) and SEUSS was initially characterized as the regulator of polar patterning in petals (Franks *et al*., 2006). This protein complex has been brought to attention in leaf polarity study because of the physical interaction between LUG and FIL, a member of the YAB protein family, based on data obtained from bioluminescence resonance energy transfer assays (Stahle *et al.,* 2009). Additionally, *fil yab3 lug* triple mutants display adaxial patterning on the abaxial surface. A gene expression analysis in the triple mutants shows that LUG may up-regulates the *YAB3* expression level and the LUG-YAB is required for the control of PHB-expressing domain. This suggests a novel interaction between LUG and YAB.

Recently, an elegant experiment was conducted to identify REV direct target genes using a ChIP-seq approach. The study also demonstrates the possible role of REV and leaf polarity establishment in adaptive development by regulating genes involved in the shade avoidance response. *TAA1* and *YUCCA5* encode enzymes in the biosynthesis pathway of auxin, which stimulates cell expansion. *HAT2, HAT3, ATHB2/HAT4* and *ATHB4* encode proteins that belong to the HD-ZIP II family and function in the shade avoidance signaling. Quantitative expression analysis shows that over-expression of REV was able to up-regulate expression of these six genes. In contrast, KAN was found to

repress the expression of *TAA1, YUC5* and *HAT2.* This result emphasizes the significance of leaf polarity establishment regulated by REV and KAN that may enable the shade avoidance response. Although our knowledge about leaf polarity establishment has been progressing, many questions still remain unanswered. For example, the identity of the meristem-generated adaxialization signal is still not known. Auxin is a phytohormone that is a potential candidate of this elusive adaxialization signal because *ARF* genes are involved with one of the leaf polarity regulators. Additionally, several genes in the auxin signaling pathway have been proposed as targets of leaf polarity regulators. On the other hand, what is the regulatory gene in Arabidopsis that is functionally equivalent to *PHAN* in leaf polarity establishment? Does this gene necessarily encode a transcription factor or a protein whose molecular function is totally different from PHAN? Characterization of a single recessive Arabidopsis mutant that displays mutant phenotypes similar to those of *phan* is needed to identify this elusive regulator. Although several transcription factors involved in leaf polarity establishment have been identified, very little is known about their target genes. Thus far, the direct binding between KAN1 and *AS2* promoter and REV and its target genes are the only demonstrated examples. Genome-wide expression and chromatin immuno-precipitation analyses are required to characterize other downstream targets of other major transcription factors. Similar to what was found in the discovery of the REV direct target genes, this may lead us to discover novel biological significance of leaf polarity in other aspects of plant growth, development and adaptation. The involvement of ribosomal proteins in leaf polarity regulation has also raised other questions. The genetic study indicates that the ribosomal protein *RPL28A* synergistically interacts with adaxial and abaxial determinants. Does this ribosomal protein function equally in the adaxial and abaxial domains? If not, what is the mechanism controlling their activity in each domain? Another question is whether ribosomal proteins directly or indirectly engage in the establishment of leaf polarity. Are the cell proliferation defects found in *ae3, ae5* and *ae7* the only factors affecting leaf polarity? Additional studies are needed to fulfill our understanding about the role of ribosomal proteins as translational machineries in establishment of leaf polarity.

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