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Perspectives on leaf dorsoventral polarity

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Abstract Leaves occur in a vast array of shapes and sizes, with complex diversity contributing to optimization of the principal function of photosynthesis. The program of development from a self-renewing stem cell population to a mature leaf has been of interest to biologists for years. Many genes involved in this process have been identified, particularly in the model eudicot Arabidopsis, so that now we have a greater understanding of mechanisms of stem cell maintenance, cell differentiation and organogenesis. One aspect of leaf development that is of particular interest is the establishment of dorsoventral polarity: the distinct adaxial (upper) and abaxial (lower) sides of the leaf. Early studies postulated conceptual models of how establishment of polarity leads to the development of planar leaves. Studies over the past decade have defined genetic details of this model, and uncovered diverse mechanisms of gene regulation that facilitate development of leaf dorsoventral polarity, including transcriptional regulation, chromatin modification, DNA modification, regulation by short RNAs and translational and post-translational regulation. This review will discuss these regulatory mechanisms in the context of leaf dorsoventrality, and will conclude with unresolved questions and areas of future research.

Keywords Dorsoventral · Leaf · Polarity

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Introduction

Leaf cells are derived from a small population of stem cells, within the shoot meristem, in the growing tip of the plant. As the leaf grows, cells divide, expand and differentiate following a program of morphogenesis that typically results in a planar organ with three main axes of polarity (Fig. 1a). Leaves of most plants have morphologically distinct adaxial and abaxial leaf tissues. In Arabidopsis, the adaxial side of the leaf faces the sun, and is more trichome-rich and a darker green, with internal cell layers organised to maximize light capture by the chloroplasts. The abaxial surface is more stomata-rich and lighter green, with internal cell layers more specialised for gas exchange (Fig. 1b). Leaf vasculature is also aligned such that xylem tissue is adaxial, while the phloem is arranged abaxially (Fig. 1c).

When leaf primordia arise on the flanks of the meristem, the adaxial surface of the leaf faces the meristem (Fig. 1a). Some of the earliest experiments demonstrating a relationship between the meristem and adaxial fate were carried out by Sussex in the 1950s (Sussex 1952, 1955). In these experiments, using exposed meristems of potato, incisions were made separating the meristem from the presumptive leaf primordium. These incisions resulted in growth of primordia distally from the meristem, but failure to produce flattened planar leaves. Instead, leaf primordia formed "centric organs", with varying degrees of vascular development. Some radial organs gained lamina growth at the distal tip, while others were completely lacking leaf blade expansion. Separation of initiating leaf primordia from the meristem using laser ablation and micro-dissection techniques also results in leaves that are radial with abaxial surface characteristics (Reinhardt et al. 2005). Furthermore, disruption of only the outermost L1 cell layer

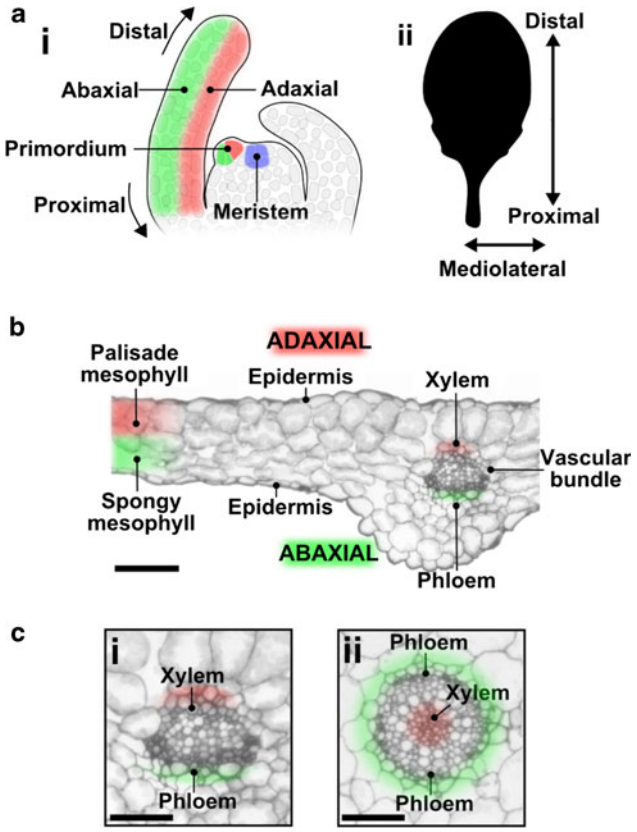

Fig. 1 Leaf dorsoventrality. **a** (*i*) Diagram representing a longitudinal section of the Arabidopsis vegetative shoot apex. (*ii*) Outline of an Arabidopsis leaf showing the mediolateral axis and proximodistal axis. **b** Transverse section through a mature Arabidopsis leaf showing tissue types. **c** (*i*) A wild type vascular bundle showing arrangement of xylem and phloem. (*ii*) An abaxialized vascular bundle with phloem surrounding xylem. *Bars* **b** 100 μm, **c** 50 μm

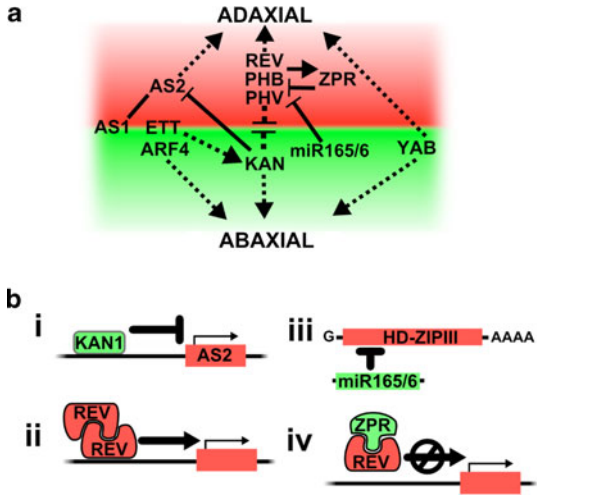

Fig. 2 Dorsoventral polarity gene interactions. **a** Scheme representing gene expression in the adaxial and abaxial domains of the leaf. Gene names are placed to represent expression either in the adaxial, abaxial or both domains. *Upper* adaxial domain, *lower* abaxial domain. *Dashed lines* represent genetic interactions; *filled lines* represent genetic and physical interactions. **b** Cartoon showing some of the molecular mechanisms controlling dorsoventral polarity gene expression. (*i*) KAN1 acts as a transcriptional repressor in the promoter of *AS2*. (*ii*) REV, depicted here as a homodimer, acts as a transcriptional activator at putative adaxial loci. (*iii*) The microRNA miR165/6 directs cleavage at the transcript of HD-ZIPIII transcription factors including *PHB*, *PHV* and *REV*. (*iv*) The ZPR protein forms a complex with REV, preventing REV from DNA binding and gene activation

in the boundary between the meristem and emerging primordia is sufficient to cause organ radialization. Together these experiments demonstrate three major points. Firstly, a signal from or contact with the meristem is necessary for the formation of adaxial leaf identity. Secondly, if adaxial identity is lost, the organ assumes abaxial identity. Finally, if adaxial identity is lost the leaf lamina does not expand, suggesting that both adaxial and abaxial specification is required for lamina outgrowth. As we discuss in this review, many genes involved in establishing this patterning have now been identified (Fig. 2a).

Transcriptional regulators

The first step towards understanding the molecular mechanism of leaf dorsoventral patterning came from studies of the *Antirrhinum* gene *PHANTASTICA* (*PHAN*), which encodes a MYB-domain transcription factor (Waites et al. 1998). Early leaves in *phan* mutants display patches of abaxial-like tissue on the adaxial side of the leaf, and around these patches ectopic lamina develops. More severely affected leaves are radial, abaxialized organs (Waites and Hudson 1995). The *phan* phenotype was pivotal in revealing a genetic basis for dorsoventral polarity that regulated leaf adaxial fate and that the juxtaposition of adaxial and abaxial domains is necessary for lamina outgrowth.

A loss-of-function mutation in the Arabidopsis *PHAN* orthologue, *ASYMMETRIC LEAVES1* (*AS1*), surprisingly does not have a strong dorsoventral polarity defect (Byrne et al. 2000), even though there appears to be conservation of biochemical function across AS1 orthologues from divergent species. *AS1* from *Cardamine hirsuta*, a close relative of Arabidopsis, complements the Arabidopsis *as1* mutation, as does the more distantly related maize orthologue *ROUGH SHEATH2* (*RS2*) (Hay and Tsiantis 2006; Theodoris et al. 2003). One conserved role for *AS1* in higher plants is repression of meristem class I KNOX homeobox transcription factor genes in developing leaf primordia, which in Arabidopsis includes *BREVIPEDICELLUS* (*BP*), *KNAT2* and *KNAT6* (Byrne et al. 2000; Ori et al. 2000; Semiarti et al. 2001). While class I KNOX genes are repressed in the simple leaf of Arabidopsis,

reactivation of KNOX genes within leaves is associated with formation of a complex dissected leaf shape (Bharathan et al. 2002; Hareven et al. 1996; Hay and Tsiantis 2006).

Another class of transcription factors central to establishment of dorsovental polarity are the Class III HD-ZIP (HD-ZIPIII) genes. Members of this gene family share a DNA binding homeodomain, a leucine-zipper that facilitates the formation of homo- and heterodimers, a predicted sterol-binding START domain and a C-terminal MEKHLA or PAS-like domain, that is related to protein domains involved in chemical sensing (Baima et al. 1995; Mukherjee and Bürglin 2006; Schrick et al. 2004; Sessa et al. 1998). HD-ZIPIII genes also have a single binding site for microRNAs miR165 and miR166 (Mallory et al. 2004; Reinhart et al. 2002; Rhoades et al. 2002). In Arabidopsis, there are five HD-ZIPIII genes, which have distinct but overlapping patterns of expression, including expression in the meristem, as well as polar expression in the adaxial region of cotyledons and leaves, and in vasculature (Baima et al. 1995; McConnell et al. 2001; Otsuga et al. 2001; Prigge et al. 2005; Zhong and Ye 1999). Recessive mutations in individual HD-ZIPIII genes do not have visible phenotypes, except for *revoluta* (*rev*) mutants, which display defects in axillary and floral meristem function (Emery et al. 2003; Otsuga et al. 2001; Prigge et al. 2005; Talbert et al. 1995; Zhong and Ye 1999). However, combined loss of *REV* with the most closely related HD-ZIPIII genes *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*) results in embryo defects, with lack of meristem function and formation of radial and abaxialized cotyledons. Semi-dominant mutations in *PHB*, *PHV, REV* and *ATHB15* (also known as *CORONA* and *INCURVATA*), which disrupt the microRNA binding site, result in ectopic expression of the mutant HD-ZIPIII gene throughout the leaf and the development of radial, adaxial leaves (Emery et al. 2003; McConnell and Barton 1998; McConnell et al. 2001; Ochando et al. 2006; Zhong and Ye 2004). Both loss- and gain-of-function mutant phenotypes of HD-ZIPIII genes support the role of these transcription factors in adaxial determination.

Several unrelated families of transcription factor genes are necessary and sufficient for abaxial fate specification, consistent with a juxtaposition model of opposing fates setting a pattern of dorsoventral polarity. KANADI genes are GARP-domain transcription factors that are expressed throughout the abaxial domain of lateral organs, in a pattern complementary to HD-ZIPIII genes *PHB*, *PHV* and *REV* (Eshed et al. 2001; Kerstetter et al. 2001). Mutation in *KAN1* causes mild dorsoventral patterning defects including upward curled leaves and precocious development of abaxial trichomes (Eshed et al. 2001; Kerstetter et al. 2001). Progressive loss of KANADI gene function, through combinations of mutations in *KAN1*, *KAN2* and *KAN3* results in more dramatic adaxialization of leaves, with abaxial ectopic lamina outgrowths that are proposed to arise from patches of adaxial tissue on the abaxial side of the leaf (Eshed et al. 2001, 2004; Izhaki and Bowman 2007). Conversely, expression of *KAN1* throughout the leaf results in meristemless seedlings with long, narrow abaxial cotyledons (Eshed et al. 2001). Together, the expression pattern of KANADI genes and phenotypes conferred by loss- and gain-of-function alleles of KANADI supports a role for these genes in promoting abaxial fate. At least part of this process may involve regulating flow and distribution of the hormone auxin. Directional polar localization of the PIN1 auxin transporter within cells of the shoot meristem generates auxin maxima necessary for organ initiation, and in the combined KANADI mutant *kan1 kan2 kan4*, ectopic lamina outgrowths on the hypocotyl are associated with ectopic PIN1 localization (Izhaki and Bowman 2007). KANADI genes may therefore contribute to organ patterning by regulating localized expression of *PIN1*.

Adaxial and abaxial leaf fates are established partly by interplay between KANADI and HD-ZIPIII genes (Izhaki and Bowman 2007). Loss of *PHB*, *PHV* and *REV* is partially suppressed by loss of *KAN1*, *KAN2* and *KAN4,* consistent with ectopic expression of KANADI genes in HD-ZIPIII mutants. Likewise, *kan* mutant phenotypes are partially rescued by loss of *PHB*, *PHV* and *REV* function. However, in both cases rescue of mutant phenotypes is incomplete, possibly due to redundancy with other family members in these two gene classes or to the activity of additional patterning components (Izhaki and Bowman 2007). The mutual repression between KANADI and HD-ZIPIII genes is likely to be indirect, and in the case of vascular patterning both components of this pathway act by influencing canalization of auxin (Ilegems et al. 2010).

KAN1 promotes abaxial fate through repression of the adaxial LOB-domain transcription factor *ASYMMETRIC LEAVES2* (*AS2*) (Iwakawa et al. 2002; Shuai et al. 2002; Husbands et al. 2007; Wu et al. 2008). *AS2* is expressed throughout initiating leaf primordia but expression becomes restricted to the adaxial region of the developing leaf primordia and is most prominent in the outermost L1 cell layer (Iwakawa et al. 2007, 2002). Mutations in *AS2* result in mildly lobed leaves and only a weak adaxial defect, similar to null mutations in *AS1*, which produces a protein partner of AS2 (Byrne et al. 2002; Iwakawa et al. 2002; Lin et al. 2003; Semiarti et al. 2001; Shuai et al. 2002; Xu et al. 2003). Ectopic expression of *AS2* in the abaxial domain of the leaf produces phenotypes comparable to loss of KANADI gene function, with outgrowths of lamina from the abaxial side of the leaf (Lin et al. 2003). The overexpression of *AS2* results in reduced expression of *KAN1*, suggesting AS2 directly or indirectly represses

KAN1. Conversely, KAN1 directly represses *AS2* in the abaxial domain of the leaf (Fig. 2b). KAN1 binds a cis-element in the promoter region of *AS2* and a mutation in this cis-element results in misexpression of *AS2* throughout cotyledon and leaf primordia, indicating this sequence is necessary for the proper spatial expression of *AS2* (Wu et al. 2008).

KANADI genes also appear to be central to the role of two closely related AUXIN RESPONSE FACTOR (ARF) genes, *ETTIN* (*ETT/ARF3*) and *ARF4*, in specification of leaf abaxial fate. Loss of *ETT* and *ARF4* do not dramatically affect leaf patterning, however, combined loss of these two genes results in abaxialized, upwardly pointing leaves with abaxial lamina outgrowths, a phenotype strikingly similar to that of *kan1 kan2* mutants (Pekker et al. 2005; Sessions et al. 1997). Loss of *ETT* function suppresses the effects of ectopic expression of *KAN1*, consistent with these genes being in a common pathway. However, KANADI genes do not regulate transcription of *ETT* and *ARF4* (Pekker et al. 2005). One possibility is that ETT and ARF4 protein function or stability is altered by loss of KANADI, an effect that may be indirect or through direct interactions between members of these gene families. *ARF4* is expressed in the abaxial domain of lateral organs, consistent with a role in abaxial fate, whereas *ETT* transcripts are detected throughout lateral organs (Pekker et al. 2005). Direct interaction with KANADI proteins would confer some specificity for ETT function in the abaxial region of the leaf.

Members of another gene family, the YABBY transcription factor family, have multiple roles, acting in leaf polarity as well as lamina outgrowth and meristem function. YABBY genes encode HMG-like proteins and interact in a complex with GRO-TUP1-like co-repressors LEUNIG and LUENIG-HOMOLOG and co-regulator SUESS (Sawa et al. 1999b; Siegfried et al. 1999; Stahle et al. 2009). In Arabidopsis, three YABBY genes, *FILAMENTOUS FLOWER* (*FIL*), *YAB2* and *YAB3* are initially expressed throughout the abaxial domain of leaves and later in development are restricted to abaxial margin regions (Sawa et al. 1999b; Siegfried et al. 1999). Single mutations in these YABBY genes do not affect leaf polarity, although in *fil* mutants floral organs are frequently radial. By contrast, combined loss of *FIL* and *YAB3* leads to partial loss of abaxial fate (Siegfried et al. 1999). Overexpression of either *FIL* or *YAB3* also results in organs with some polarity defects, with abaxial characteristics of epidermal cells on both upper and lower leaf surfaces, consistent with YABBY genes functioning in abaxial fate (Sawa et al. 1999a; Siegfried et al. 1999). However, combining mutations in *FIL*, *YAB3* and *YAB5* leads to formation of leaves that display abaxial features even though these genes are expressed on the abaxial side of the leaf. This indicates YABBY genes have a non-cell-autonomous role on adaxial cell fate (Stahle et al. 2009). YABBY-mediated signalling also impacts on the maintenance of the shoot meristem, even though these genes are expressed in the domain of the leaf furthest from the meristem. Tissue specific expression studies demonstrate FIL and YAB3 are not mobile and the non-cell-autonomous effect of YABBY genes is due to short distance movement of downstream signalling factors (Goldshmidt et al. 2008).

Modification of chromatin state

Transcriptional regulation is modulated by changes in chromatin state induced both by post-translational modification of histones and by genomic DNA methylation. Setting chromatin state during differentiation may serve as a mechanism for stable expression or repression of genetic programmes essential to leaf development (Roudier et al. 2009). One key step during leaf initiation is repressing meristem identity KNOX genes and, for simple leaves, maintaining repression throughout leaf development. At least some repressors of KNOX genes are involved in chromatin remodelling and act via components of the dorsoventral patterning pathway. The chromatin remodelling factor HIRA has been identified as a protein partner of AS1 and maize RS2 (Phelps-Durr et al. 2005). Homozygous *hira* mutants are embryo lethal, whereas weak co-suppression lines have some similarity to *as1,* displaying short petioles, curled asymmetric lamina and leaf lobe formation. As in *as1*, reduced expression of *HIRA* results in ectopic expression of the KNOX genes *BP* and *KNAT2* in leaves. However, plants with reduced *HIRA* in the *as1* background show a synergistic interaction, indicating *AS1* and *HIRA* likely also act independently in leaf patterning (Phelps-Durr et al. 2005). Histone deacetylation appears to have a more pronounced effect on polarity, acting in parallel with *AS1* and *AS2* in adaxial fate determination. Polarity defects of *as1* and *as2* are enhanced by chemical inhibitors of histone deacetylation, and RNAi knockdown of two *HISTONE DEACETYLASE* genes, *HDT1/HD2A* and *HDT2/HD2B*, in an *as2* background results in mutants producing abaxialized filamentous and trumpet-shaped leaves (Ueno et al. 2007).

In addition, expression of *AS1* appears to be regulated by *GENERAL TRANSCRIPTION FACTOR GROUP E6* (*GTE6*), which encodes a predicted chromatin remodelling bromodomain protein (Chua et al. 2005). Loss of *GTE6* results in reduced levels of *AS1*, and *AS1* is elevated in *35S:GTE6* plants. Regulation of *AS1* appears to be direct as GTE6 binds in the *AS1* promoter (Chua et al. 2005). It is possible that *GTE6* functions to maintain expression of *AS1*

in leaves and AS1 together with HIRA repress KNOX genes. Such interactions may help to maintain stable repression of an indeterminate gene expression programme subsequent to leaf initiation.

Regulation of mRNA stability by small RNAs

The discovery of small RNAs and related pathways opened an exploding field in molecular biology and development. Post-transcriptional regulation of gene expression through small RNAs involves generation of small RNAs from double stranded or hairpin precursor RNAs, through the action of RNase III endonuclease DICER proteins. ARGONAUTE family proteins bind and target small RNAs to specific mRNA transcripts, which are subsequently inactivated by cleavage of the transcript or by translational inhibition (Chen 2009).

In Arabidopsis, small RNAs regulate several families of genes involved in leaf dorsoventral patterning. HD-ZIPIII genes all have a conserved binding site for two microRNAs, miR165 and miR166, which mediate cleavage of HD-ZIPIII transcripts (Fig. 2b) (Mallory et al. 2004; Reinhart et al. 2002; Rhoades et al. 2002). Mutations in the HD-ZIPIII microRNA binding site leads to expression of the mutant allele throughout leaf primordia and development of adaxialized leaves, indicating a critical role for post-transcriptional regulation of HD-ZIPIII gene expression (Emery et al. 2003; McConnell et al. 2001; Ochando et al. 2006; Zhong and Ye 2004). miR165 is encoded by two genes and miR166 is encoded by seven genes. Although the activity of each copy is yet to be demonstrated through loss-of-function mutations, miR165 is expressed in the abaxial domain of leaves, supporting a role for these microRNAs in patterning leaf polarity through repressing HD-ZIPIII expression (Kidner and Martienssen 2004). In the gain-of-function *jabba-1D* mutant, overexpression of *miR166g* results in down-regulation of some but not all HD-ZIPIII genes. *jabba-1D* (*jab-1D*) mutants have radial leaves, although unexpectedly these leaves are adaxialized due to up-regulation of *REV*. Possibly the *jab-1D* phenotype reflects complex interactions between HD-ZIPIII genes (Williams et al. 2005).

Post-transcriptional regulation of *ETT* and *ARF4* is mediated by another class of small RNAs called *trans*-acting siRNAs (ta-siRNAs). ta-siRNAs are derived from non-coding transcripts of *TAS* loci. *TAS* transcripts initially undergo microRNA cleavage and are then processed by RNA-DEPENDENT RNA POLYMERASE6 (RDR6) and SUPPRESSOR OF GENE SILENCING3 (SGS3) to form double stranded RNA. The resulting double stranded RNA is processed by DICER-LIKE4 (DCL4) to produce small ta-siRNAs that target specific transcripts for cleavage. ta-siRNAs derived from *TAS3* direct cleavage of *ETT* and *ARF4* transcripts (Adenot et al. 2006; Allen et al. 2005; Gasciolli et al. 2005; Peragine et al. 2004; Vazquez et al. 2004; Xie et al. 2005; Yoshikawa et al. 2005). Of the known ta-siRNA processing factors, *AGO7* specifically targets *TAS3* due to selective binding of miR390 (Montgomery et al. 2008). Surprisingly there are two miR390 binding sites in *TAS3*. The 3' complementary site is highly conserved and AGO7-miR390 directs cleavage at this site, whereas the 5′ site contains mismatches to miR390 that prevent cleavage (Axtell et al. 2006; Montgomery et al. 2008). Conversion of the 5′ site to a cleavable sequence inactivates *TAS3* indicating that this site serves an essential but as yet unknown role in generation of *TAS3* ta-siRNAs.

Components of the ta-siRNA pathway have accelerated phase change, which is the transition from production of juvenile leaves to adult leaves. This phenotype is largely due to altered ta-siRNA mediated regulation of *ETT* and *ARF4* (Hunter et al. 2003; Peragine et al. 2004; Yoshikawa et al. 2005). Expression of a ta-siRNA cleavage-resistant *ETT* results in accelerated phase change, similar to ta-siRNA pathway mutants (Fahlgren et al. 2006; Hunter et al. 2006). Further increases in the dose of *ETT*, when *ETT* or cleavage-resistant *ETT* are expressed in an *rdr6* mutant results in highly lobed leaves. How this integrates in leaf patterning pathways is still to be established but increased leaf lobing also results when ta-siRNA pathway mutants are combined with *as1* or *as2* mutants (Garcia et al. 2006; Li et al. 2005; Xu et al. 2006). The enhancement of *as1* and *as2* leaf pattern defects by ta-siRNA mutants is due, in part, to increased levels of *ETT*. Furthermore, the abaxial gene *FIL*, is up-regulated when *as1* is combined with ta-siRNA mutants. Loss of *ETT* in these mutants suppresses *FIL* misexpression, indicating that *ETT* may act in leaf dorsoventral polarity via regulation of *FIL* (Garcia et al. 2006).

Limited spatial expression of components of the TAS3 pathway appears to result in restricted production of *TAS3* ta-siRNAs (Chitwood et al. 2009; Garcia et al. 2006; Schwab et al. 2009). Most notable is the expression of *TAS3* and *AGO7*, which are localized to the L1 and subepidermal layers of the adaxial side of the leaf. Processing of *TAS3* in these tissue layers results in accumulation of a *pETT:ETT-GUS* translational fusion reporter on the abaxial side of the leaf, unlike the endogenous transcript, which is broadly expressed in the leaf (Chitwood et al. 2009; Pekker et al. 2005; Schwab et al. 2009). ta-siRNAs are mobile and may have a role in short range signalling (Tretter et al. 2008). Therefore it is possible that ta-siRNAs establish gradients of *ETT* and *ARF4* expression that help to define the boundary between adaxial and abaxial domains of the leaf (Chitwood et al. 2009; Schwab et al. 2009).

Regulation of protein levels

In plants, transcripts targeted by microRNAs are typically inactivated by cleavage while in animals miRNAs regulate gene expression largely by repressing translation of target transcripts. A small number of microRNA targets have been shown to be translationally repressed in plants, but it may be that microRNA-mediated translational repression is relatively common in plants (Aukerman and Sakai 2003; Brodersen et al. 2008; Chen 2004; Gandikota et al. 2007).

ARGONAUTE1 (*AGO1*), the key effector of microRNA gene silencing in plants, and *PINHEAD/ZWILLE/AGO10* (*PNH/ZLL*) are both required for specifying leaf dorsoventral patterning. Mutations in *AGO1* result in loss of abaxial fate and development of adaxial leaves (Kidner and Martienssen 2004). By contrast, mutations in *PNH/ZLL* cause the formation of radial, abaxial organs (Lynn et al. 1999; Moussian et al. 1998). The opposing roles of *AGO1* and *PNH/ZLL* in dorsoventral polarity may be due to differences in expression domains of these two genes and differences in function (Mallory et al. 2009). In leaves, *AGO1* is ubiquitously expressed, whereas *PNH/ZLL* expression is limited to the adaxial region of the leaf (Lynn et al. 1999; Tucker et al. 2008). Expression of *AGO1* under the control of the *PNH/ZLL* promoter results in suppression of most but not all *ago1* phenotypes, indicating the expression domain of *AGO1* is important for function. Expression of *PNH/ZLL* via the *AGO1* promoter does not suppress *ago1* mutant phenotypes, indicating different activities of these two ARGONAUTE proteins (Mallory et al. 2009). AGO1 binds microRNAs and mediates target transcript cleavage, whereas this has not yet been demonstrated for *PNH/ZLL*. However, both *AGO1* and *PNH* appear to have a role in translational repression. AGO1 protein and microRNAs are associated with polysomes, and this association is sensitive to mRNA degradation and to translation inhibition (Lanet et al. 2009). Consistent with polysome association, several microRNA targets have substantially higher protein levels relative to transcript levels in *ago1* mutants (Brodersen et al. 2008; Lanet et al. 2009). AGO1 itself is regulated by translational repression mediated by *PNH/ZLL* and this may account for opposing roles of these genes in leaf polarity (Mallory et al. 2009). Precisely how translational regulation is mediated and how the two post-transcriptional mechanisms interact is yet to be determined, but further understanding of this process might reveal new layers of regulation in dorsoventral patterning.

Another mechanism involved in translational regulation of dorsoventral patterning genes may involve the ribosome. Ribosomes are large ribonucleoprotein complexes that catalyze polypeptide chain formation. In eukaryotes, ribosomes consist of a 40S small subunit and a 60S large subunit. The 80 different cytoplasmic ribosomal proteins contributing to both subunits in Arabidopsis are encoded by small gene families of 2–5 functional members (Barakat et al. 2001). Ribosomal proteins have multiple functions in development but mutations in a number of ribosomal protein genes appear to have a role in leaf adaxial fate (Byrne 2009; Pinon et al. 2008; Yao et al. 2008). Single mutations in ribosomal protein genes have mild leaf shape alterations, including pointed lamina with pronounced marginal serrations (Fujikura et al. 2009; Ito et al. 2000; Pinon et al. 2008; Rosado et al. 2010; Van Lijsebettens et al. 1994; Yao et al. 2008). However, ribosomal protein mutants *piggyback* (*pgy*) and *asymmetric leaves1/2 enhancer* (*ae*) result in enhancement of polarity defects of *as1* (Pinon et al. 2008; Yao et al. 2008). In combination with *as1*, mutations in *PGY* ribosomal protein genes confer ectopic lamina outgrowths on the adaxial side of the leaf. In severe cases, mutations in *AE* ribosomal protein genes together with *as1* or *as2* produce pin- or trumpet-shaped leaves. These polarity phenotypes are further enhanced by mutations in *REV* and are suppressed by mutations in KANADI genes. The relatively mild loss of adaxial fate in *as1 pgy1* mutants is associated with increased levels of KANADI genes, although transcriptional down-regulation of HD-ZIPIII genes is not detected (Pinon et al. 2008). Ribosomal protein genes may act either by promoting HD-ZIPIII gene expression, possibly regulating HD-ZIPIII protein levels or activity, or by indirectly repressing KANADI expression. Specific target genes in this pathway and how these are regulated by the ribosome remain to be determined.

Protein abundance can be regulated by targeted proteolysis and unwanted proteins can be marked through a catalytic cascade with a degradation signal that is recognized by a large effector complex called the proteasome. Protein turnover through proteasome degradation appears to influence leaf dorsoventrality. An enhancer of *as1* and *as2*, *asymmetric leaves enhancer3* (*ae3*), conditions development of abaxialized pin-like and lotus leaves in combination with *as1* and *as2* (Huang et al. 2006). In these double mutants radial leaves are associated with elevated levels of abaxial transcription factors and down-regulation of *REV*. Furthermore, when *ae3* is combined with either *rev* or *rdr6*, both of which affect adaxial fate, leaves develop as radial abaxial organs (Huang et al. 2006). Although mutations in other proteasome subunit components have similar outcomes in enhancing leaf polarity defects of *as2*, it is yet to be determined whether these synergistic interactions are due to overlap of proteasome function with other leaf polarity pathway genes.

Pairing proteins

Protein–protein interactions are an emerging theme in key regulatory steps required for dorsoventral patterning. AS1 physically interacts with AS2 in a complex that binds to promoters of the KNOX genes *BP* and *KNAT2* to repress expression of these genes in leaves. Interestingly, domains of *AS1* and *AS2* expression overlap only in the earliest stages of leaf initiation, when both genes are expressed throughout the leaf primordium. *AS1* expression becomes restricted to the region between the adaxial and abaxial domains, while *AS2* expression becomes confined to the adaxial region of the developing leaf primordia and is most prominent in the L1 cell layer (Byrne et al. 2000; Iwakawa et al. 2007, 2002). Although loss of *AS1* or *AS2* does not result in prominent dorsoventral defects, ectopic expression of *AS2* with either a 35S promoter or an *AS1* promoter results in strong leaf adaxialization, a gain of function phenotype that is dependent on a functional copy of *AS1*. By contrast, overexpression of *AS1* does not result in leaf adaxialization, suggesting the function of AS1-AS2 protein interaction in leaf polarity is principally determined by localization of *AS2* (Lin et al. 2003; Theodoris et al. 2003; Xu et al. 2003).

HD-ZIPIII genes are regulated post-transcriptionally by microRNAs and, in addition, HD-ZIPIII activity is regulated through direct interaction with proteins encoded by *LITTLE ZIPPER* (*ZPR*) genes (Kim et al. 2008; Wenkel et al. 2007). There are four *ZPR* family genes in Arabidopsis. ZPR proteins are related to HD-ZIPIII proteins through similar leucine zipper domains, which are important for protein dimerization, but ZPR proteins lack the DNA binding homeodomain present in HD-ZIPIII proteins. Like HD-ZIPIII genes, *ZPR* genes are expressed in the adaxial domain of the leaf. Binding of ZPR to HD-ZIPIII proteins likely changes HD-ZIPIII function and interferes with DNA binding (Fig. 2b) (Wenkel et al. 2007). Overexpression of *PHB* and *REV* leads to elevated *ZPR* expression, while triple *phb phv rev* mutants have reduced expression of *ZPR*. Overexpression of *ZPR3* leads to phenotypes similar to loss-of-HD-ZIPIII function including development of abaxialized leaves and meristem termination, whereas double mutant *zpr3 zpr4* plants have a pleiotropic phenotype including disrupted meristem and ectopic meristem formation but no apparent leaf polarity defects. The role of ZPR in leaf polarity may be masked by redundancy with other member of this family. Thus HD-ZIPIII and ZPR proteins function in negative feedback regulation where HD-ZIPIII transcription factors activate *ZPR* transcription, and interaction of ZPR proteins with HD-ZIPIII proteins inhibits HD-ZIPIII function (Kim et al. 2008; Wenkel et al. 2007).

Concluding remarks

Understanding leaf dorsoventral patterning has been greatly expanded through molecular genetics and investigations into the molecular basis of patterning mechanisms have led to a model where opposing interactions of adaxial and abaxial factors establish a planar leaf. The first genes to fit this model were found to be factors that regulate transcription, such as *ASYMMETRIC LEAVES1*, *ASYMMETRIC LEAVES2*, and the HD-ZIPIII and KANADI genes. Additional factors that interact directly or indirectly with these genes to regulate their function have since been found, adding molecular details to the conceptual model of leaf dorsoventral polarity. As many of the genes involved in dorsoventral patterning regulate transcription, it will be important to identify direct targets and regulatory networks governed by these genes. The role of signaling molecules, in particular auxin and potentially small RNAs, in leaf dorsoventral polarity and integration with meristem function are aspects of morphogenesis to be fully resolved. While significant progress has been made on understanding dorsoventral patterning, much less is known on genetic control of mediolateral symmetry and proximodistal patterning of the leaf, and on understanding how molecular control of patterning integrates with factors that influence physical stresses associated with growth. These constitute important areas of research that will lead to a more complete understanding of leaf development. Analysis of these pathways in different species will also provide an understanding of genetic pathway redundancy and how this influences phenotype diversity and environmental plasticity.

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