Adaxial–Abaxial Polarity: The Developmental Basis of Leaf Shape Diversity

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Summary: Leaves of flowering plants are diverse in shape. Part of this morphological diversity can be attributed to differences in spatiotemporal regulation of polarity in the upper (adaxial) and lower (abaxial) sides of developing leaves. In a leaf primordium, antagonistic interactions between polarity determinants specify the adaxial and abaxial domains in a mutually exclusive manner. The patterning of those domains is critical for leaf morphogenesis. In this review, we first summarize the gene networks regulating adaxial–abaxial polarity in conventional bifacial leaves and then discuss how patterning is modified in different leaf type categories. genesis 52:1–18, 2014. © 2013 The Authors. Genesis Published by Wiley Periodicals, Inc.

Key words: leaf type; bifacial leaf; unifacial leaf; peltate leaf

INTRODUCTION

Leaves are lateral organs that have diversified during the evolution of flowering plants. Although the flat shape of typical leaves is efficient for their primary function as the site of photosynthesis, flowering plants have also evolved morphologically distinct leaves with novel functions. For example, some climbing plants produce leaf tendrils to support their body, and some carnivorous plants develop pitcher-shaped leaves to capture small animals as a nutrient source (Bell and Bryan, 2008).

Leaves are produced at the tips of leaf-bearing branches called shoots (reviewed in Groff and Kaplan, 1988). The shoot apex contains stem cells and proliferative tissues, together called the shoot apical meristem (SAM) (Esau, 1977). The first morphogenetic signature of leaf initiation is a periclinal cell division, which forms a new cell wall parallel to the organ surface, in the inner tissues of the SAM periphery (e.g., Cunninghame and Lyndon, 1986; Tepfer, 1960). Along with subsequent cell proliferation, leaf primordia acquire organ polarities. Adaxial–abaxial polarity is determined based on position relative to the SAM. The cells close to the SAM differentiate into the adaxial domain, and those far from the SAM become the abaxial domain, generating a bifacial structure in the leaves (Fig. 1). Leaf margins are formed in the boundary of the adaxial and abaxial domains (Steeves and Sussex, 1989). In the early stage of leaf blade development, proliferative activity is induced at the marginal region (Esau, 1977). As leaf development proceeds, cell proliferation is gradually replaced by cell differentiation, including increases in cell size (reviewed in Tsukaya, 2013). Finally, leaf primordia develop into mature leaves with two anatomically distinct faces, the adaxial and abaxial sides...
corresponding to the upper and lower sides, respectively (Fig. 2). Leaves with only bifacial parts are termed conventional bifacial leaves, whereas leaves exhibiting different facialities are classified into other leaf type categories (Fig. 3) (reviewed in Franck, 1976).

The developmental mechanisms of conventional bifacial leaves have been well studied in model plants including Arabidopsis (*Arabidopsis thaliana*) and maize (*Zea mays*) (Fig. 4), and those of other leaf types are being progressively elucidated in different plant species. In this review, we summarize what is known mainly about the development of conventional bifacial leaves and then discuss mechanisms underlying the diversification of leaf shape, with emphasis on adaxial-abaxial polarity.

**TERMINOLOGY RELATED TO ADAXIAL-ABAXIAL POLARITY**

The concept of adaxial-abaxial polarity refers to asymmetry in tissue differentiation associated with position relative to the SAM (reviewed in Kaplan, 2001). In this article, we use adaxial/abaxial “side” to describe positional relationships, whereas adaxial/abaxial “character” is reserved to denote gene expression and tissue differentiation, characteristic of the adaxial/abaxial side of conventional bifacial leaves (Fig. 1). Adaxial/abaxial “domain” indicates leaf tissue with adaxial/abaxial characters. The adaxial/abaxial “surface” is the surface layer of the domains. These designations are helpful in dissecting the faciality of diverse leaf types, especially in those with complicated patterns of adaxial and abaxial domains.

**ASYMMETRIC TISSUE DIFFERENTIATION WITH REGARD TO ADAXIAL-ABAXIAL POLARITY**

Leaf tissues are organized into a layered structure and differentiate asymmetrically along the adaxial-abaxial axis. The adaxial epidermis of Arabidopsis is composed of jigsaw puzzle-shaped pavement cells, with more trichomes, and fewer stomata than found on the abaxial epidermis (Bowman, 1994). The inner tissues of the leaf also display polarity. The adaxial inner tissue contains palisade mesophyll, composed of elongated cells perpendicular to the surface that form a cell layer optimized for light capture...
In the abaxial side, spongy mesophyll has loosely packed cells surrounded by air space that facilitates photosynthetic gas exchange through abaxial stomata. This arrangement enables the adaxial light-harvesting tissue to be in the vicinity of the photosynthetic substrate, namely carbon dioxide, supplied from the abaxial tissue. As such, the layered organization serves as an adaptation for efficient photosynthesis (Braybrook and Kuhlemeier, 2010; Kaplan, 2001).

Vascular tissue also has adaxial–abaxial polarity in leaves. In Arabidopsis, vascular bundles differentiate in a collateral arrangement with xylem and phloem on the adaxial and abaxial sides, respectively (Figs. 2c and 3a,b). In addition, some plants develop bicolateral vascular bundles, which have additional phloem on the adaxial side (Dengler and Kang, 2001; Esau, 1977).

Asymmetric tissue differentiation has been subject to structural modification during adaptation to a specific habitat. For example, bifacial leaves of Eucalyptus spp. (Fig. 2b) have palisade mesophyll on both the adaxial and abaxial sides. Despite the anatomical symmetry of the mesophyll layers, Eucalyptus leaves have adaxially positioned xylem and abaxially positioned phloem (Fig. 2d), and therefore are considered to represent a form of bifacial leaves with reduced asymmetry. In natural
habitats, two faces of adult leaf blades of *Eucalyptus globulus* tend to face east and west (James and Bell, 2000), and these so-called equifacial *Eucalyptus* leaves are considered to utilize incident light from the sun at low angle by both the adaxial and abaxial assimilatory tissues (Kaplan, 2001).

**FIG. 3.** Typical examples of different leaf types. (a) Schematic representation of a transverse section of a conventional bifacial leaf. Leaf appearance, toluidine blue-stained transverse section, and vascular polarity are shown. The positions at which the sections were prepared are indicated by the dashed lines. (b) Conventional bifacial leaf of Arabidopsis. (c) Terete leaf of Juncus setchuenensis var. effusoides. (d) Ensi-form leaf of Acorus gramineus. (e) Phylloide of Acacia coreyi. (f) Spherical leaf of Senecio rowleyanus. (g) Leaf flattened along the median plane in Se. crassissimus. (h) Peltate leaf of Hydrocotyle vulgaris. (i) Keeled pitcher leaf of Sarracenia purpurea. (j) Bi-keeled pitcher leaf of Heliamphora nutans. (k) Pitcher leaf of Nepenthes alata. (l) Compound leaf with a terminal tendril in Pisum sativum. (m) Foliated leaf of Herbertia amoena. (n) Ericoid leaf of Phyllodoce caerulea. Solid circles in the section contours indicate vascular bundles: blue, xylem; yellow, phloem. Scale bars denote 5 mm and 100 μm for leaf appearance and cross sections, respectively. Abbreviations: SAM, shoot apical meristem; pe, petiole; la, lamina; lb, lateral vascular bundle; mb, main vascular bundle; ke, keel; ap, aperture; lu, pitcher lumen; bl, basal laminar portion; te, tendril; li, lid; st, stipule; ra, rachis; ll, leaflet; pm, primary margin; mr, midrib.

**SPECIFICATION OF ADAXIAL DOMAIN**

During the establishment of adaxial–abaxial polarity, leaf primordia are hypothesized to receive mobile signal(s) from the SAM. Surgical experiments have shown that a leaf primordium that has been physically
FIG. 4. Schematics representing development of leaves with different shapes in relation to the growth activity at the adaxial–abaxial boundary. Cross-sectional views of leaf primordia at the establishment of adaxial–abaxial polarity (left), blade outgrowth (middle), and maturation (right) are shown. (a) Conventional bifacial leaf, like those of wild-type Arabidopsis. (b) Weak phenotype of phantastica (phan) mutant in snapdragon (Waites and Hudson, 1995) and knock-down phenotype of Nicotiana sylvestris PHAN ortholog (McHale and Koning, 2004b). (c) Maize milkweed pod1 mutant (Candela et al., 2008). (d) Wild-type prophyll in maize (Johnston et al., 2010). (e) Severe phenotype of phabulosa-1d in Arabidopsis (McConnell and Barton, 1998). (f) Naturally occurring terete leaf (Yamaguchi et al., 2010) or severely compromised leaf of phan in snapdragon (Waites and Hudson, 1995). (g) Ensiform leaf of Juncus prismatocarpus (Yamaguchi et al., 2010). See text for detail. Blue and yellow lines denote the adaxial and abaxial surfaces in developing leaves, respectively. Green in (d) indicates expanded adaxial domain. Black lines show transectional contours of mature leaves. SAM indicates the position of the shoot apical meristem. Purple arrows show growth direction. The primary and secondary growth axes are indicated by 1° and 2°, respectively, in (d). ND, not determined.

The first insight revealing a genetic connection between adaxial–abaxial polarity and leaf morphogenesis came from analysis of the loss-of-function mutant phantastica (phan) in Antirrhinum majus (snapdragon). PHAN encodes a MYB family transcription factor of ASYMMETRIC LEAVES1 /ROUGH SHEATH2 /PHANTASTICA (ARP) orthologous group and functions to promote adaxial characters (Byrne et al., 2000; Waites et al., 1998; Waites and Hudson, 1995). The phan mutant produces a series of abaxialized leaves wherein the adaxial side exhibits a mosaic of adaxial and abaxial characters. Blade-like outgrowths called lamina ridges are formed at the ectopic boundaries of the adaxial and abaxial domains (Fig. 4b). Waites and Hudson (1995) proposed that the adaxial–abaxial boundary induces lamina growth activity and that both adaxial and abaxial surfaces are required for lamina formation. Similar phenotypes have been observed in loss-of-function mutants of ARP genes from other eudicot species (Kim et al., 2003a, 2003b; McHale and Koning, 2004b; Tattersall et al., 2005; Zoulia et al., 2012). Similarly, some ornamental plants (e.g., Xanthosoma atrovirens and...
Three HD-ZIP III family genes, PHABULOSA et al. bifacial leaves. differentiates among plant species with conventional the regulatory mechanism of adaxial–abaxial patterning factors that function in specification of the adaxial homeodomain-leucine zipper (HD-ZIP III) transcription domain (Emery et al., 1998; Timmermans et al., 1999; Tsiantis et al., 1999). These indicate that the regulatory mechanism of adaxial–abaxial patterning differentiates among plant species with conventional bifacial leaves.

Extensive genetic screening subsequently identified several factors involved in polarity establishment (Fig. 1c). In Arabidopsis, AS1 and its structurally unrelated interactor AS2 positively regulate class III homeodomain-leucine zipper (HD-ZIP III) transcription factors that function in specification of the adaxial domain (Emery et al., 2003; Fu et al., 2007; McConnell et al., 2001; Semiarti et al., 2001; Xu et al., 2003). Three HD-ZIP III family genes, PHABULOSA (PHB), PHAVOLUTA (PHV), and REVOLUTA (REV), redundantly function on the adaxial side to promote adaxial differentiation (Emery et al., 2003; McConnell and Barton, 1998; Prigge et al., 2005; Talbert et al., 1995). Their messenger RNAs (mRNAs) harbor a microRNA recognition site and their expression domains are tightly regulated by miR165/166-guided mRNA cleavage (Mallory et al., 2004; Rhoades et al., 2002; Tang et al., 2003). Plant small RNAs are able to move cell-to-cell (Dunoyer et al., 2010; Molnar et al., 2010). In accord with this, miR165/166 precursors are predominantly transcribed in the abaxial side (Miyashima et al., 2013; Yao et al., 2009) and are suggested to move toward the adaxial side (Juarez et al., 2004a). miR165 is known to act non-cell-autonomously in roots, where it is transferred through plasmodesmata (Carlsbecker et al., 2010; Miyashima et al., 2011; Vatén et al., 2011). Defects in ARGONAUTE1 (AGO1), which functions in miR165/166 loading into the RNA-induced silencing complex (RISC), bring about leaf adaxialization (Bohmert et al., 1998; Kidner and Martienssen, 2004). By contrast, another AGO protein, ZWILLE/PINHEAD/AGO10, binds to miR165/166 without inducing target cleavage, thereby acting as a decoy to inactivate miR165/166 in the adaxial side (Liu et al., 2009; Zhu et al., 2011). Mutations in HD-ZIP III genes at the miRNA binding site cause ectopic expression of miRNA-resistant mRNA, giving rise to adaxialized leaves in Arabidopsis, maize, and Nicotiana sylvestris (Fig. 4c) (Emery et al., 2003; Juarez et al., 2004a, 2004b; McConnell and Barton, 1998; McConnell et al., 2001; McHale and Koning, 2004a).

KANADIs, ETTIN/AUXIN RESPONSE FACTOR 3 (ETT/ARF3), and ARF4 are expressed in the abaxial side and cooperatively function in abaxial specification (Eshed et al., 1999; Kerstetter et al., 2001; Pekker et al., 2005; Sessions et al., 1997; Sessions and Zambryski, 1995). KANADI genes encode GARP family of transcription factors that abaxially expressed in Arabidopsis, Oryza sativa (rice), and maize (Candela et al., 2008; Kerstetter et al., 2001; Yan et al., 2008; Zhang et al., 2009). In Arabidopsis, KANADIs act antagonistically to AS2 and HD-ZIP III genes, repressing adaxialization in the abaxial side (Emery et al., 2003; Iwasaki et al., 2013; Wu et al., 2008). Abaxialized phenotype of KANADI1 (KAN1) misexpression is suppressed in ett/arf3 mutant (Pekker et al., 2005), which is concordant with the formation of a KANADI and ETT/ARF3 protein complex (Kelley et al., 2012). ARF4 is a sister gene of ETT/ARF3 and is found in all of previously examined angiosperms except rice, maize, and their relatives (Finet et al., 2013, 2010). In Arabidopsis and tomato, ARF4 mRNA localizes to the abaxial side, while ETT/ARF3 mRNA expresses also in the adaxial side as well as the abaxial side (Pekker et al., 2005; Yifhar et al., 2012). Despite the broad expression of mRNA, its protein localizes to the abaxial side in wild-type Arabidopsis (Chitwood et al., 2009). An open reading frame in the 5′ region of ARF3/ETT mRNA modulates the translation of ETT/ARF3, although its spatial regulation in leaf tissues remains unknown (Nishimura et al., 2004, 2005; Rosado et al., 2012).

Accumulation and abaxial localization of ETT/ARF3 and ARF4 is regulated by tasi-RARF, a member of a class of endogenous small RNA called trans-acting small-interfering RNA (tasiRNA) (Adenot et al., 2006; Allen et al., 2005; Garcia et al., 2006; Williams et al., 2005). While TAS3a, which is a precursor noncoding RNA, is expressed only in the adaxial side, the mature 21-nucleotide tasiR-ARF shows a wider distribution as a result of intercellular movement toward the abaxial side, forming a gradient of distribution (Chitwood et al., 2009; Garcia et al., 2006; Nogueira et al., 2007; Schwab et al., 2009). Arabidopsis mutants defective in tasiRNA biogenesis overaccumulate ARF3/ETT mRNA and misexpress its protein throughout leaf primordia (Adenot et al., 2006; Chitwood et al., 2009), although such mutants do not show polarity phenotypes similar to those found in rice and maize mutants (Nagasaki et al., 2007; Nogueira et al., 2007). However, the tasiRNA biogenesis mutants with as1 or as2 background form abnormal leaves in which adaxial surface harbors patches of adaxial and abaxial identities (Li et al., 2005; Xu et al., 2006). This suggests that both pathways redundantly function in Arabidopsis. In addition, biogenesis of the tasiR-ARF itself is positively
regulated by the AS1-AS2 protein complex through upregulation of miR390 and RDR6 expression (Iwasaki et al., 2013). Simulation of the small RNA-mRNA interaction illustrated that diffusible tasiR-ARF could form the sharp boundary of target ARF expression domain (Levine et al., 2007). However, in maize, tasiR-ARF biogenesis is required for proper expression level of ARF3 but not for abaxial localization of its transcript, suggesting that tasiR-ARF-independent mechanism polarizes ARF3 expression in this species (Douglas et al., 2010).

Together, the analyses of genetic interactions reveal a generalized view of the regulatory relationships between adaxial and abaxial factors: adaxially localized transcription factors repress abaxial factors and vice versa (Fig. 1c). Accumulating evidence indicates that small RNAs are the key players for intercellular signaling in the transcriptional network across the two domains. The work to date supports a model in which antagonistic interactions coordinate the mutually exclusive specification of adaxial and abaxial cell fates and thereby facilitate precise tissue differentiation.

**BLADE FORMATION IN BIFACIAL LEAVES**

After the establishment of adaxial–abaxial polarity, a dome-shaped primordium develops into a flat structure partly as a result of active cell division at the marginal region, where the adaxial and abaxial domains are juxtaposed (Fig. 4a) and characteristic margin cells develop (Esau, 1977). Interaction between the adaxial and abaxial domains is necessary for the onset of the directed growth that forms leaf blades. Although the mechanism by which directed growth activity is initiated remains largely unknown, analyses of YABBY and WUSCHEL-RELATED HOMEOBOX (WOX) genes have provided evidence linking adaxial–abaxial polarity and the growth activity.

Expression of YABBY genes *FILAMENTOUS FLOWER/YABBY1* (*FIL/YAB1*) and *YAB3* marks the abaxial domain and marginal regions of primordial leaves in Arabidopsis (Sawa et al., 1999; Siegfried et al., 1999). Abaxial expression is conserved in a snapdragon ortholog *GRAMINIFOLIA* (Golz et al., 2004). In maize and rice, the YABBY orthologs show adaxial and uniform expression, respectively (Dai et al., 2007; Juarez et al., 2004b), although ancestral expression pattern in angiosperms was inferred to be abaxial (Yamada et al., 2011). In Arabidopsis, YABBY genes are upregulated by KANADI, *ETT/ARF3*, and *ARF4* (Eshed et al., 2004; Garcia et al., 2006), and, in turn, *FIL/YAB1* and *YAB3* promote the expression of *KAN1* and *ARF4*, forming positive feedback loops (Bonaccorso et al., 2012; La Rota et al., 2011). In the absence of all YABBY activities, leaf primordia establish adaxial–abaxial polarity but fail to initiate lamina outgrowth (Sarojam et al., 2010). Moreover, loss of YABBY activity abolishes ectopic outgrowths in the *kan1 kan2* double mutant (Eshed et al., 2004). These findings indicate that YABBY genes mediate the induction of growth activity related to adaxial–abaxial polarity.

YABBY genes positively regulate a member of the WOX gene family, *PRESSED FLOWER* (*PRS*), which is expressed in the leaf margin and promotes blade outgrowth (Fig. 1c) (Nakata et al., 2012). *PRS* cooperatively functions with *WOX1*, and the *prs wox1* double mutant exhibits a narrower-leaf phenotype in Arabidopsis (Nakata et al., 2012; Vandebussche et al., 2009). Similarly, mutants disrupted in *PRS* or *WOX1* orthologs in other species show compromised leaf blades (Cho et al., 2013; Ishiwata et al., 2013; McHale, 1993; Nardmann et al., 2004; Tadge et al., 2011; Vandebussche et al., 2009). *lamina1* (*lam1*), a loss-of-function mutant of a WOX ortholog from *Nicotiana sylvestris*, retains the capacity to specify blade founder cells, but fails to commence blade outgrowth, suggesting that *LAM1* has a role in blade formation after adaxial–abaxial specification (McHale, 1993).

The WOX genes act in leaf blade formation differently in plant lineages. *WOX1* are conserved in eudicots but is absent from rice and maize (Nardmann et al., 2007; Zhang et al., 2010). In Arabidopsis, *prs wox1* double mutant displays reduced cell proliferation in the marginal region of leaf primordium (Nakata et al., 2012), while the compromised leaf blades in loss-of-function mutant of maize *PRS* orthologs are largely attributed to the failure in founder-cell recruitment into leaf blades from SAM (Nardmann et al., 2004; Scanlon et al., 1996, 2000; Scanlon and Freeling, 1997). This may reflect the difference in the developmental mechanisms of leaf blade initiation between these species.

The *PRS* and *WOX1*-dependent blade outgrowth is, in part, mediated by an as-yet-unidentified mobile signal(s) processed by the cytochrome P450 KLHU/CYP78A5 (*KLU*) in Arabidopsis (Nakata et al., 2012). *KLU* non-cell-autonomously promotes cell division activity in aerial organs including leaves (Anastasiou et al., 2007). The loss-of-function mutant of *KLU* exhibits reduced organ sizes, and a series of *KLU*-restored lines show different organ sizes in correlation with the restored *KLU* expression level. This dose-dependent effect of *KLU* attributed changes in the longevity of the cell proliferative stage, but not in the growth rate.

Auxin appears to be another signal acting in blade formation (Wang et al., 2011), independent of *KLU* (Anastasiou et al., 2007). Multiple loss-of-function mutants of the *YUCCA* (*YUC*) auxin-biosynthetic genes show defective blade outgrowth, raising the possibility that auxin participates in the regulatory network for directed growth activity (Wang et al., 2011). *YUCCA*-dependent growth activity is ectopically activated in *as2 rev* and *kan1 kan2* double mutants, indicating that auxin biosynthesis is coordinated by adaxial and abaxial factors.
UNIFACIAL LEAVES AND THEIR ANALOGS

Nonequivalent development of adaxial and abaxial tissues gives rise to mature leaf morphology different from that of conventional bifacial leaves (Fig. 4). Abaxialization usually causes a cylindrical leaf type with radialized symmetry, referred to as unifacial (reviewed in Franck, 1976). Vascular bundles in these leaves are arranged in a radial pattern with phloem outside and xylem inside (section 2 in Fig. 3c). Unifacial leaves with cylindrical leaf blades, called terete leaves, are prevalent in monocots. While the distal portion of monocotyledonous terete leaves is fully abaxialized, a basal part usually retains adaxial characters (section 1 in Fig. 3c).

Ensiform leaves, another type of unifacial leaf, have a flat blade along the median plane, yet exhibit radial symmetry in terms of vascular polarity (e.g., Acorus gramineus, Fig. 3d) (Arber, 1921). In these leaves, the ETT/ARF3 ortholog is expressed throughout the entire surface of the flattened blade, whereas the PHB ortholog is confined to the adaxial side of the basal part, consistent with their anatomical features including vascular polarity (Yamaguchi et al., 2010). Terete and ensiform leaves have evolved repeatedly in monocots (Rudall and Buzgo, 2002).

Ensiform leaves develop into flattened structures in the absence of adaxial-abaxial juxtaposition, suggesting that they utilize an alternative mechanism for blade outgrowth (Yamaguchi and Tsukaya, 2010). The YABBY gene DROOPING LEAF (DL) was recently identified as a regulator of this process. In ensiform leaves of Juncus prismatocarpus, incipient leaf primordia acquire proliferative activity in the adaxial side, where DL is expressed (Fig. 4g) (Yamaguchi et al., 2010). This morphogenetic event is called adaxial growth (Kaplan, 1975), and it is not observed in cylindrical unifacial leaves of J. wallachianus (Fig. 4f). Consistent with this, DL expression is not detected in the adaxial side of J. wallachianus leaves (Yamaguchi et al., 2010). Results from crosses between the two species suggest that the DL locus regulates the specific growth activity of ensiform leaves. After the adaxial growth, proliferative activity is induced in both margins of a leaf primordium, where PRS orthologs are expressed (Fig. 4g) (Yamaguchi et al., 2010). In conventional bifacial leaves of rice, DL is expressed in the medial part of primordia, where the midrib develops, and promotes midrib thickening (Yamaguchi et al., 2004), suggesting that the expression patterns and gene functions are partly conserved between ensiform leaves of J. prismatocarpus and conventional bifacial leaves of rice.

Terete-like and ensiform-like leaves also evolved in eudicots, although their exact faciality has not been determined conclusively. Acacia phyllodes are composed mainly of an abaxialized rachis-petiole that is flattened along the median plane like ensiform leaves (Fig. 3e) (Arber, 1918, 1921; Boke, 1940; Kaplan, 1970). These phyllodes are produced by adaxial growth that causes the primordium to become a planar structure (Boke, 1940). Unlike in monocot ensiform leaves, bidirectional growth does not occur in Acacia phyllodes, and unilateral adaxial growth continues until maturation. In another example from eudicots, the succulents Senecio rowleyanus and S. crassissimus produce terete-like and ensiform-like leaves, respectively (Fig. 3f,g). Primordia in both species first develop into transversely flat structures like conventional bifacial leaves. Subsequently, by adaxial growth, they are rounded out to be terete-like or flattened along the median plane to be ensiform-like (Hillson, 1979; Ozerova and Timonin, 2009). Growth patterns in this process suggest that the adaxial surface expands in the mediolateral direction, but the adaxial surface does not. As a result, the adaxial surface dominates at maturity and this type of leaf is classified as subunifacial (Ozerova and Timonin, 2009). Subunifacial leaves also evolved in some species of Peperomia including P. dolabriformis (Kaul, 1977). The molecular mechanisms of ensiform-like leaf development remain unknown in eudicots.

PELTATE LEAVES AND THEIR ANALOGS

Peltate leaves are widespread in at least 40 flowering plant families (Gleissberg et al., 2005). While conventional bifacial leaves have a lamina that is basifixed to a bifacial petiole, peltate leaves have a unifacial petiole attached to the abaxial side of the lamina, forming a shield-like structure (Fig. 3h) (Franck, 1976). The peltate structure is hypothesized to enable efficient light capture on an erect petiole (Givnish and Vermeij, 1976). In the emerging stage, peltate leaf primordia are indistinguishable from those of conventional bifacial leaves (Hagemann and Gleissberg, 1996). Subsequently, the “cross zone” characteristic of peltate leaf development is formed (Fig. 5). The cross zone corresponds to the basal portion of the incipient lamina, where leaf margins are congenitally fused (Troll, 1932).

A glimpse into the molecular mechanisms of peltate leaf development was provided by the pbam mutant, in which conventional bifacial leaves are sometimes converted into peltate leaves (Waites and Hudson, 1995). Abaxialization of a proximal portion of the leaf has been proposed to be the cause of the pbam peltate-leaf phenotype (Waites and Hudson, 1995). Arabidopsis and other model plants that normally produce conventional bifacial leaves also can produce peltate leaves as a result of mutations that impair adaxial-abaxial patterning (summarized in Table 1, often referred to as “lotus” or “trumpet” leaves in the original reports). Two types of peltate leaves, epipeltate and hypopeltate, can be differentiated based on the arrangement of the adaxial and abaxial surfaces (reviewed in Franck, 1976). Epipeltate
leaves show a distal-adaxial basal-abaxial arrangement and arise in loss-of-function mutants of the adaxial determinants \textit{AS1}, \textit{AS2}, and HD-ZIP III genes (Prigge \textit{et al.}, 2005; Sun \textit{et al.}, 2002; \textit{Xu et al.}, 2002, 2003).

The hypopeltate leaf type exhibits the opposite polarity: distal-abaxial and basal-adaxial. Gain-of-function mutants of HD-ZIP III genes develop hypopeltate leaves due to adaxialization of the basal part of the leaf.
Peltate leaves can be funnel-shaped or follicle-shaped. Such leaves are termed epipactidiate or hypoasidiate leaves depending on their adaxial-aboral pattern, similarly as described in the previous paragraph (Franck, 1976). Epipactidiate leaves are often associated with specialized functions, especially in carnivorous plants (Fig. 3i–k). Three phylogenetically independent families, the Nepenthaceae, the Sarraceniaceae, and the Cephalotaceae, form epipactidiate leaves that function as pitfall traps to capture small animals (Albert et al., 1992; Juniper et al., 1989). Hypopeltate or hypoasidiate leaves are also widely distributed in flowering plants including *Toecca* and *Maieria* in the Melastomataceae, *Cassiope* in the Ericaceae, *Dischidia* in the Apocynaceae, and *Celmisia* in the Asteraceae (Franck, 1976).

All examples described above are peltate leaves with undivided leaf blades, but compound leaves, which are composed of a set of leaflets, can also be peltate. In peltately palmate compound leaves, leaflets are attached to the tip of a unifacial rachis, which is structurally equivalent to the petiole of simple peltate leaves. Kim et al. (2003a) examined PHAN expression in eight compound-leaved species sampled across eudicots, and found that the expression domain correlates to the adaxial fate and leaflet initiation sites in peltately palmate compound leaves. In non-peltately palmate compound leaves and pinnate compound leaves, PHAN genes are expressed in the adaxial side from the tip to the basal portion of leaf primordia including the rachis. By contrast, PHAN expression marks a distal portion of peltately palmate leaf primordia where leaflets initiate. Those expression patterns are reminiscent of the developmental model for simple peltate leaves (Waites and Hudson, 1995). Knock-down of the PHAN ortholog in *Solanum lycopersicum* (tomato) converts pinnate compound leaves into peltately palmate compound leaves (Kim et al., 2003a). A mutation in the PHAN ortholog of *Pisum sativum* (pea) causes peltate leaflets rather than peltately palmate compound leaves to form (Tattersall et al., 2005). Taken together, those results suggest that similar expression changes in the adaxial and abaxial determinants underlie peltation in simple leaves, compound leaves, and leaflets.

**TENDRILS**

Climbing plants have tendrils, which are twining organs filamentous in shape (Fig. 3i) (Bell and Bryan, 2008). Leguminous species form leaf tendrils, which fully lack a laminar portion and are interpreted as abaxialized leaflets or rachises (Tattersall et al., 2005). *Tendrill-less* (*Tl*) encodes a structurally unusual Class I HD-ZIP protein and was isolated as a regulator of terminal tendril formation in pea (Hofer et al., 2009). Loss-of-function *Tl* mutants develop a leaflet in place of the terminal tendril. *Tl* mRNA accumulates in tendril primordia at the stage when organ fate is determined, indicating that *Tl* functions in the establishment of tendril identity including the absence of blade outgrowth (Hofer et al., 2009). *Tl* expression is upregulated by *LATHYROIDES*, a *WOX1* ortholog in pea (Zhuang et al., 2012). *Tl* orthologs have been identified in tendrilled legumes including *Vicia* spp., *Lens culinaris*, and *Lathyrus odoratus*, but not in the nontendrilled plants *Arabidopsis* and *Medicago truncatula* (Hofer et al., 2009), suggesting that *Tl* function is specific to tendril formation.

**ECTOPTIC LAMINAS**

Protrusive laminar structures arise in various leaf types. These ectopic laminas can be circular (i.e., closed margin) or linear (i.e., open margin). In the latter case, the structure is specifically referred to as a keel. Ectopic laminas increase the morphological complexity of leaves. For example, foliated leaves of some species in the Iridaceae bear keels in an iterative manner (e.g., *Herbertia amoenia*, Fig. 3m) (Arber, 1921), and, as a result, the keels comprise a large portion of those leaves. Ectopic laminas can also be related to novel functions. A pair of keels on the abaxial surface of some ericoid leaves form a humid chamber immediately outside stomatal pores (e.g., *Phyllocooe caerulea*: Fig. 3n), which is considered to be advantageous in dry environments (Böcher, 1981; Hagerup, 1946; Kron et al., 2002). The keels on pitcher leaves in the carnivorous plant genus *Sarracenia* can compensate for the inefficient photosynthetic activity (Ellison and Gotelli, 2002) and reinforce structural strength of pitcher leaves (Fig. 3i) (Juniper et al., 1989).

An ectopic lamina can be bifacial or unifacial. Accumulating evidence suggests that the initiation of bifacial ectopic laminas depends on growth activity at the adaxial-abaxial boundary. The *milkweed pod1* (*mpw1*) loss-of-function mutant of a KANADI gene in maize develops bifacial ectopic laminas on the abaxial surface (Candela et al., 2008). In the leaves of the *mpw1* mutant, adaxial characters are ectopically conferred to the abaxial surface and growth activity is induced, presumably in response to the ectopic adaxial-abaxial boundary (Fig. 4c) (Candela et al., 2008; Johnston et al., 2010). More evidence for the importance of the adaxial-abaxial boundary came from analysis of the maize prophyll, the first leaf of a shoot. In the Poaceae, prophyls are intrinsically bi-keeled and are formed by congenital fusion of two primordia (Arber, 1923). Based on the expression pattern of a HD-ZIP III gene in maize, Johnston et al. (2010) proposed that the bifacial keels initiate due to the new adaxial-abaxial boundaries generated by transient expression of adaxial
determinant(s) on the abaxial surface (Fig. 4d). Opposite to mpw1 in maize, knock-down of a PHAN ortholog in Nicotiana sylvestris results in the formation of bifacial laminas on the adaxial surface (Fig. 4b) (McHale and Koning, 2004b). Sporadic abaxial characters on the adaxial side may underlie this ectopic lamina formation (McHale and Koning, 2004b). Because bifacial ectopic laminas are frequently formed over or on a flank of veins, Kidner and Wrigley (2010) hypothesized that the prolonged expression of adaxial–abaxial determinants like HD-ZIP IIIs and KANADIs in vascular elements can contribute to reversing the previously determined polarity and therefore to establishing a new adaxial–abaxial boundary on the founder region of bifacial ectopic laminas.

Compared with bifacial ectopic laminas, unifacial ectopic laminas are poorly characterized and their developmental mechanisms remain unclear.

COMBINATIONS OF DIFFERENT ADAXIAL–ABAXIAL PATTERNING WITHIN A LEAF

A leaf is divided into distinct portions along the proximal–distal axis: a leaf base, a petiole, and a lamina, as well as each leaflet in the case of compound leaves. Differential regulation of adaxial–abaxial patterning in proximal–distal segments is a source of leaf shape diversity. For example, the pitcher, tendril, and basal laminar portion of Nepenthes leaves presumably correspond to the lamina, petiole, and leaf base of conventional bifacial leaves, respectively (Franck, 1976; Kaplan, 1973; Troll, 1932), and show distinct adaxial-abaxial patterning judged from vascular bundle orientation (Fig. 3k). In another example, monocotyledonous unifacial leaves of both terete and ensiform types have bifacial and unifacial portions in their basal and distal parts, respectively (Fig. 3c,d).

Disturbance of adaxial-abaxial polarity can have local effects, giving rise to different faciality within a leaf. The pea mutant crispa and the tomato mutant wiry6 exhibit phenotypes in which a portion of the leaflets are peltate despite others retaining conventional bifacial structure (Kim et al., 2003b; Tattersall et al., 2005). Some Arabidopsis mutants develop malformed leaves wherein a peltate distal portion is connected with a proximally located bifacial structure by a midrib without a leaf blade (Table 1) (Kojima et al., 2011; Zhong and Ye, 2004). Structurally similar leaves spontaneously arise in Codiaeum variegatum var. pictum f. appendiculatum (Fig. 6a) (De Vries, 1905) and Camellia japonica cv. “Kinglyobatsu” (Fig. 6b). As these examples come only from phenotypes observed in various mutants and cultivars, solid evidence for their adaptive significance is yet to be identified. However, these observations indicate that genetic changes related to adaxial-abaxial patterning can result in further segmentation or addition of novel segments in leaves from a broad range of taxa.

REARRANGEMENT OF THE EXPRESSION DOMAINS OF ADAXIAL–ABAXIAL DETERMINANTS

In some cases, the expression of adaxial-abaxial determinants are not static. In Arabidopsis leaf development, FIL/YAB1 shrinks its expression domain in parallel with the expansion of miR165/166-free domain (Tameshige et al., 2013). Stamen development provides an explicit example of expression rearrangement. Leaves and floral organs are both formed laterally and share part of the same developmental gene network, including the interactions between adaxial and abaxial determinants. Among lateral organs in flowering plants, stamens are substantially differentiated in terms of morphology. Based on analyses of rice stamen morphogenesis, Toriba et al. (2010) proposed that growth activity is induced at the expression boundary of the adaxial and abaxial determinants to form pollen sacs, and that the expression patterns are rearranged during stamen development. Initially, the expression domains of ETT/ARF3...
and PHB orthologs occupy the abaxial and adaxial sides, respectively, as in conventional bifacial leaves. However, as stamen development proceeds, the expression domains are rearranged into a complex pattern that generates four expression boundaries where pollen sacs initiate. This rearrangement does not disturb the complementary patterns: an ETT/ARF3 ortholog comes to occupy both adaxial and abaxial corners, while the PHB ortholog localizes to two lateral corners between the two expression domains of the ETT/ARF3 ortholog (Toriba et al., 2011). The rod-like lemma mutant shows compromised expression patterns of ETT/ARF3 and PHB orthologs, and a reduced number of pollen sacs (Toriba et al., 2010). These observations substantiate the idea that expression rearrangement of polarity determinants mediates the development of nonplanar organs like stamens.

Another example of expression rearrangement is found in the peltate-leaved species Tropaeolum majus. In leaf primordia, TmFIL mRNA is localized to the abaxial side at an early stage, reminiscent of FIL/YAB1 localization in conventional bifacial leaves of Arabidopsis (Gleissberg et al., 2005). This expression pattern is concordant with the observation that peltate leaves of this species arise from fully bifacial primordia (Hagemann and Gleissberg, 1996). Later, the TmFIL expression domain expands into the adaxial side of the petiole (Gleissberg et al., 2005) and the initially bifacial petiole is secondarily compensated by additional growth to form radially symmetric structure (Hagemann and Gleissberg, 1996). This suggests that the TmFIL expression domain is rearranged to generate a new adaxial-abaxial boundary at the cross-zone, where growth activity is induced (Fig. 5).

Although the mechanism by which expression domains are rearranged remains unclear, it is clear that expression rearrangement contributes to the morphological diversification of lateral organs.

**PERSPECTIVE**

Extensive analyses using model plants have identified regulatory genes that are involved in developmental patterning, increasing our knowledge of the gene networks governing leaf morphology in flowering plants. Expression changes in the adaxial-abaxial regulators have been revealed to be a source of leaf shape diversification. However, the genetic alterations underlying these changes in expression are unknown. Recent forward-genetic approaches established in some non-model plants have begun to yield results promoting our understanding of the genetic mechanisms of leaf type evolution, however. In Begonia, peltate-leaved species and those with conventional bifacial leaves are compatible, and crossing experiments have revealed that the peltateness is a semidominant trait (DeWitte et al., 2011; Neale et al., 2006). In Juncus, crosses between terete-leaved species and ensiform-leaved species showed that the DL locus governs leaf flatness in ensiform leaves (Yamaguchi et al., 2010). Such approaches offer the possibility of identifying causal genetic changes in leaf type evolution.

Advances in massive sequencing technology make it possible to identify the loci responsible for given phenotypes in nonsequenced genomes (e.g., Weber et al., 2013). In addition, the establishment of widely applicable gene silencing techniques facilitates analyses of gene function (reviewed in Becker and Lange, 2010). With the advantage of these new technologies, the mechanisms and processes of leaf type evolution appear likely to be unraveled in the near future.

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