Journal of Plant Biology, June 2007, 50(3) : 282-290 **REVIEW**

Developmental Processes of Leaf Morphogenesis in *Arabidopsis*

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The leaf is a suitable subject with which to study plant morphogenesis because of its diversity of shape. Although mechanisms for leaf initiation and lateral morphogenesis have been suggested, the exact means for determining shape remain unclear. Many genes involved in those developmental processes have now been identified. Here, we summarize the early events in the genetic regulation of *Arabidopsis* leaf formation, including initiation, dorsoventrality, and the spatial and temporal control of cell proliferation and enlargement. We focus on recent progress within the model plant *Arabidopsis*, placing special emphasis on our own findings.

Keywords: Arabidopsis thaliana, dorsoventrality, leaf initiation, leaf morphogenesis, polar cell expansion

BRIEF HISTORY OF STUDIES OF LEAF DEVELOPMENT IN *ARABIDOPSIS*

Many mutants of *Arabidopsis* with alterations in their leaf morphology were isolated in the 1960s and 1970s (e.g., Rédei, 1962; Barabas and Rédei, 1971). In the early 1990s, some leaf morphogenesis mutations were described from studies of T-DNA mutagenesis (van Lijsebettens et al., 1991), but attempts to characterize these new mutants had not yet started. In the mid-1990s, anatomical analyses were begun on the development of cotyledons and leaves in the *Arabidopsis* 'Columbia' wild-type strain, with researchers finding that cotyledons could serve as a model system for studies of leaf morphogenesis (e.g., Tsukaya et al., 1994). In addition, van Lijsebettens et al. (1994) used T-DNA insertional mutagenesis to determine that a mutation in the S18 ribosomal protein locus caused the *pointed first leaves* (*pfl*) phenotype. Lincoln et al. (1994) also isolated and characterized *KNAT1* (*knotted*-like from *Arabidopsis thaliana1*) from the *Arabidopsis* genome. This gene is a homolog of the maize *Kn1* gene. Tsuge et al. (1996) genetically characterized the *angustifolia* (*an*) and *rotundifolia* (*rot*) mutants; they elucidated the developmental genetic regulation of two-dimensional growth of leaf blades and proposed that leaf shape is controlled by two independent, polarity-dependent cell elongation processes.

Since the late 1990s -- and with the progress made in the *Arabidopsis* genome project -- many genes involved in leaf development have been cloned in that genus. Our successful cloning of the *ROT3* gene, which is involved in the expansion of leaf cells in the longitudinal direction, was a pioneering study toward clarifying the functioning of genes in leaf development (Kim et al., 1998b). In addition, Micol and colleagues began their genetic analysis of leaf ontogeny in *Arabidopsis* by performing a large-scale screening for mutants with abnormal leaves (Berna et al., 1999).

From the early 2000s, the use of genetics approaches in isolating genes increased for investigating leaf development. For example, we have cloned the *AN* gene, which is involved in the expansion of leaf cells in the leaf-width direction, and have found that this gene encodes one of several members of the C-terminal binding protein (CtBP) family that act as transcriptional co-repressors in animals (Kim et al., 2002). The *AN* gene modulates the polarity of cell growth by controlling the arrangement of cortical microtubules in leaf cells (Kim et al., 2002).

Many recent studies of *Arabidopsis* leaf development, including the establishment of dorsoventrality, symmetry, and flat morphology, have elucidated the mechanisms for leaf-shape control in more detail. Genes that influence leaf shape and function during these processes are summarized in Table 1.

In this review, we focus on genetic regulation in *Arabidopsis thaliana*, including early events in leaf initiation, dorsoventrality, and other aspects of the spatial and temporal balance between cell proliferation and enlargement, with special emphasis on results from our own studies. Other recent reviews have discussed the onset of leaf initiation, phyllotaxy, other aspects of primary morphogenesis, and the compensation of cell enlargement in leaf expansion (Tsukaya, 2003, 2006; Hay et al., 2004; Fleming, 2005a, b, 2006; Carraro et al., 2006; Kim and Cho, 2006); these topics are not discussed here.

DEVELOPMENTAL PROCESSES OF LEAF MORPHOGENESIS

Initiation of leaf primordia

Early control of leaf development relies on controlling initiation at the shoot apical meristem (SAM), which is located at the growing tip and is self-renewing via the activity of stem cells (Steeves and Sussex, 1989). The initiation of leaf primordia requires the repression of the KNOX (Class-I KNOTTED1-like homeobox) domain of homeodomain proteins by ASYMMETRIC LEAVES1 (AS1; Byrne et al., 2000), AS2 (Semiarti et al., 2001), BLADE-ON-PETIOLE1 (BOP1; Ha et al., 2003), SERRATA (SE; Prigge and Wagner, 2001), and PICKLE (PKL; Rider et al., 2004) in the region of the

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Table 1. Genes involved in developmental process of leaf morphogenesis.

Gene	Classification	Function on leaf shape: phenotypes	References
Leaf initiation			
KNOTTED1(KN1)[a]	*KNOX* gene in maize	dominant gain-of-function mutation: knotted and altered leaves	Kerstetter et al., 1997
		loss-of-function mutation: extra vegetative leaves	
SERRATA(SE)	C2H2-type, zinc finger protein	loss-of-function mutation: serrated leaf margin	Prigge and Wagner, 2001
PICKLE(PKL)	CHD3-chromatin remodeling factor	loss-of-function mutation: pickle roots	Rider et al., 2004
PINFORMED1(PIN1)	auxin efflux facilitator	loss-of-function mutation: naked inflorescence stem	Hay et al., 2004
SHOOT MERISTEMLESS (STM)	*KNOX* gene in *Arabidopsis*	loss-of-function mutation: shoot meristemless	Long et al., 1996
KNAT1,2,6	*KNOX* gene in *Arabidopsis*	35S-*KNAT1* plants: lobed leaves	Lincoln et al., 1994
PHANTASTICA (PHAN)[b]	MYB gene of *Antirrhinum*	loss-of-function mutation: narrow and altered leaves	Waites et al., 1998
ASYMMETRIC LEAVES1 (AS1)	MYB gene *of Arabidopsis*	loss-of-function mutation: lobed leaves with short petioles	Byrne et al., 2000
AS2	leucine-zipper motif protein	loss-of-function mutation: lobed leaves	Semiarti et al., 2001
BLADE ON PETIOLE (BOP)	BTB/POZ domain protein	loss-of-function mutation: lobed leaves and blades on petioles	Ha et al., 2003, 2004
Establishment of dorsoventrality			
ARGONAUTE1 (AGO1)	PIWI and PAZ domain	loss-of-function mutation: narrow and pointed leaves	Bohmert et al., 1998
PHABULOSA (PHB)	homeodomain-leucine zipper	dominant gain-of-function mutation: trumpet-shaped leaf, adaxialized leaf	McConnell et al., 2001
PHAVOLUTA (PHV)	homeodomain-leucine zipper	dominant gain-of-function mutation: altered and adaxialized leaf	McConnell et al., 2001
REVOUTA (REV)	homeodomain-leucine zipper	dominant gain-of-function mutation: outgrowth of leaves	Otsuga et al., 2001
YAB1/FIL	zinc finger, HMG box-like domain	35S-*FIL* plants: altered and abaxialized leaves	Sawa et al., 1999
KAN1,2,3	GARP domain	35S-*KAN1* plants: narrow and abaxialized leaves	Kerstetter et al., 2001
DEFORMED ROOTS AND LEAVES1(DRL1)	putative ATP/GTP binding motif	loss-of-function mutation: narrow leaves and rod-like leaves	Nelissen et al., 2003
PINHEAD/ZWILLE (PNH)		35S-PNH plants: ectopic meristem formation	Newman et al., 2002
Cell division in leaf expansion processes			
cdc2aAt	Cdc2 kinase	dominant negative mutation: normal-shaped leaves with reduced cell numbers	Hemerly et al., 1995
CycB1	B-type cyclin	35S-*CycB1* plants: larger leaves	Donnelly et al., 1999
CycD3;1	D-type cyclin	35S-*CycD3;1* plants: small leaves	Dewitte et al., 2003
ICK1/KRP1	cyclin-dependent kinase inhibitor	35S-*ICK1* plants: serrated and small leaves	Wang et al., 2000
ICK2/KRP2	cyclin-dependent kinase inhibitor	35S-*ICK2* plants: serrated and small leaves	
AINTEGUMENTA (ANT)	*APETALA2*-like family	loss-of-function mutation: narrow and pointed leaves	Mizukami and Fischer, 2000
		35S- *ANT* plants: larger leaves	
CURLY LEAF (CLF)	polycomb-group gene	loss-of-function mutation: narrow and small leaves	Kim et al., 1998a
POINTED FIRST LEAF2 (PFL2)	ribosomal protein S13-homolog	loss-of-function mutation: narrow and pointed leaves	Ito et al., 2000
ROTUNDIFOLIA4 (ROT4)	novel small peptide	dominant mutation: round leaf blades and short petioles	Narita et al., 2004
ARGOS	novel auxin-inducible protein	35S-AGO plants: larger organs	
		loss-of-function mutation: reduced organ size	Hu et al., 2003

Table 1. Continued.

Gene	Classification	Function on leaf shape: phenotypes	References
JAGGED(JAG)	zinc finger transcription factor	loss-of-function mutation: narrow and shredded floral organs	Ohno et al., 2004
PEAPOD(PPD)	plant-specific putative DNA binding protein	35S-*PPD* plants: smaller and flat leaves	White, 2006
STRUWWELPETER (SWP)	homeoprotein activity mediator	loss-of-function mutation: small, finger-shaped or serrated leaves	Autran et al., 2002
ANGUSTIFOLIA3 (AN3)	putative transcription factor	loss-of-function mutation: narrow leaves	Horiguchi et al., 2005; Kim and Kende, 2004
ARGOS-LIKE (ARL)		35S-*ARL* plants: larger cotyledons and leaves	Hu et al., 2006
Polar cell elongation in leaf expansion processes			
ANGUSTIFOLIA (AN)	transcriptional co-repressor (CtBP)	loss-of-function mutation: narrow leaves with altered trichomes	Kim et al., 2002
ROTUNDIFOLIA3 (ROT3)	cytochrome P450 (CYP90C1)	loss-of-function mutation: round leaf blades and short petioles	Kim et al., 1998b, 1999, 2005
LONGFOLIA1, 2 (LNG1,2)	novel protein	35S-*LNG1*: narrow and long organs	Lee et al., 2006
MicroRNA			
AGO1	miR165/ miR166	potential targets of miRNA are *PHB/PHV/REV*	Kidner and Martienssen, 2004
JAW	miR319	dominant gain-of-function mutation: serrated leaves with curvature	Palatnik et al., 2003
		potential targets of miRNA are *TCP2/TCP4*	

[a]Gene isolated from *Zea mays*.
[b]Gene isolated from *Antirrhinum majus*.

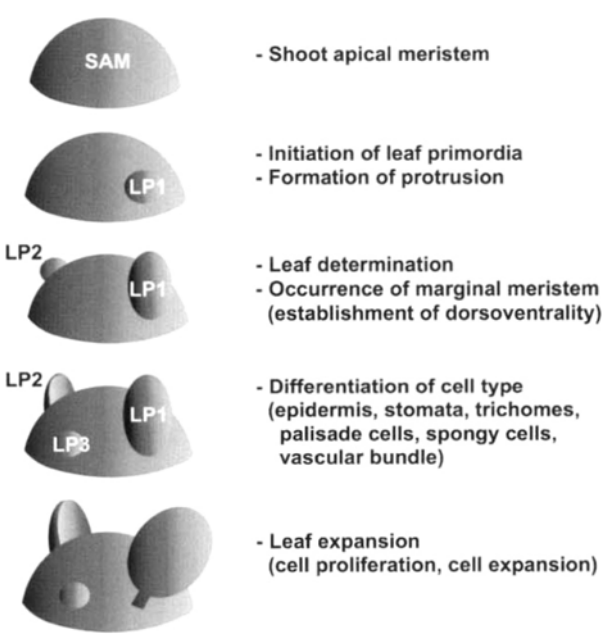

Figure 1. Developmental processes during leaf morphogenesis. Early control of leaf shape relies on controlling leaf initiation at shoot apical meristem (SAM), changes in rates and planes of cell division, and polarity-dependent differentiation of leaf cells. After initiation of leaf primordium (LP), formation of a protrusion and determination of LP (establishment of dorsoventrality) occur in early stage. Subsequent shaping of leaves during process of expansion depends on correlation between cell division and elongation.

leaf initial cells (Fig. 1). These KNOX transcription factors also interact with BEL-like homeodomain proteins and repress *AtGA20ox1* in the biosynthesis of gibberellic acid (GA) in the SAM (Jasinski et al., 2005). This suggests that the repression of GA activity by those transcription factors is a key component in meristem functioning and leaf primordia development. In contrast, GA suppresses *KNOX* mis-expression phenotypes in the leaves of 35S:*KNAT1* transgenic plants (Hay et al., 2004), implying that transfer of the KNOX/GA regulatory module from the meristem to the leaf may contribute to the generation of diverse leaf morphologies observed in higher plants. Hay et al. (2006) have suggested that the down-regulation of KNOX expression in initiating leaf primordia may require the local accumulation of both auxin and AS1. Furthermore, auxin flux controlled by the expression of *PINFORMED1* (*PIN1*) in the outer cell layer of the SAM promotes the formation and positioning of leaf primordia (Hay et al., 2004). In addition, Jasinski et al. (2005) have proposed that the KNOX function for meristem activity in the SAM is mediated by coordinated regulation of reduced GA-signaling and increased cytokinin (CK) levels. Thus, CK may promote the deactivation of GA at the boundary between leaves and the SAM in response to KNOX activity, thereby confining the activity of GA to the differentiating leaf primordia (Fig. 1).

Determination of the leaf: establishment of dorsoventrality

One common feature of most lateral organs is that they have proximal–distal ("proximal" is near the attached end; "distal" is farthest from the attached end) and abaxial–adaxial ("abaxial" is away from the meristem; "adaxial" is adjacent to the meristem) polarity of asymmetry (Kim and Cho,

2006). These polarities, established relatively early on, are defined relative to the SAM (Steeves and Sussex, 1989). Shortly after the initiation of the leaf initial, the radial symmetric primordia (Fig. 1) flatten in a plane parallel to the meristem periphery and soon display the dorsal–ventral or abaxial–adaxial polarity of asymmetry due to the asymmetrical distribution of cell types in the mature organ. Differentiation of the marginal meristem is tightly linked to dorsoventrality (Fig. 1). Furthermore, the differentiation of epidermis and palisade cells on the adaxial side of leaves and that of stomata and spongy cells on the abaxial side creates polarized leaves (Fig. 1). The adaxial side is specialized for the efficient capture of sunlight, whereas the abaxial side is specialized for gas exchange. Inner leaf tissues, such as the mesophyll or vascular tissues, are also polarized. Several genes associated with the establishment of dorsoventrality are summarized in Table 1.

PHABULOSA (*PHB*), *PHAVOLUTA* (*PHV*), and *REVOLUTA* (*REV*), which encode Class-III homeodomain/Leu zipper transcription factors, regulate adaxial–abaxial polarity in *Arabidopsis* (McConnell et al., 2001; Otsuga et al., 2001). Semi-dominant gain-of-function mutations in *PHB* and *PHV* cause a dramatic transformation of abaxial leaf fates into adaxial leaf fates, and result in the formation of adaxialized leaves that are rod-like or trumpet-shaped (Fig. 2; McConnell et al., 2001). The *phb* mutants are also defective in their vascular tissues, entirely lacking a vascular strand or possessing single xylem elements in rod-like leaves. The *phv* mutation is associated with a phenotype very similar to that of *phb1-d* plants. The *pinhead/zwille* (*pnh*) mutant exhibits a defect in leaf dorsoventrality, similar to that of the *phb-1d* mutant (Lynn et al., 1999). Restricted expression of *PNH* to the leaf adaxial cells suggests that *PNH* is required for some aspect of adaxial leaf development (Lynn et al., 1999).

In contrast, members of the gene families *YABBY* (*YAB*; encoding putative transcription factors with a zinc finger motif and a helix-loop domain) and *KANADI* (*KAN*; encoding GARP transcription factors) regulate adaxial–abaxial polarity by specifying abaxial cell fate (Sawa et al., 1999; Siegfried et al., 1999; Kerstetter et al., 2001). Although *yab3* mutants do not show the aberrant vegetative phenotype, *yab3 fil* double mutants have ovate leaves (Siegfried et al., 1999). Ectopic expression of the *YAB* family causes the ectopic differentiation of abaxial cell types in leaves, suggesting that the expression of these genes in the adaxial regions is sufficient to cause epidermal tissues to differentiate with an abaxial cell fate (Siegfried et al., 1999).

Several recent studies have suggested that miRNA controls leaf polarity by repressing the *PHB/PHV/REV* target genes (Kidner and Martienssen, 2004). Interestingly, the miRNA miR165/166, which accumulates in the abaxial domain of the primordium, is thought to regulate expression levels of the target genes (*PHB/PHV*) by the direct breakdown of transcripts for the formation and fate of adaxial cells (Kidner and Martienssen, 2004). This implies that miRNA-mediated control of leaf morphogenesis plays important roles in the variation in leaf polarity.

In *ASYMMETRIC LEAVES1* (*AS1*) of *Arabidopsis*, which encodes the *PHANTASTICA* (*PHAN*) ortholog of *Antirrhinum* (the MYB transcription factor; Waites et al., 1998), mutations induce the formation of lobed leaves and vascular defects (Fig. 2; Byrne et al., 2000; Semiarti et al., 2001). Interestingly, *as1* mutations in an *erecta* background result in rod-like leaves similar to those of *phan* mutants of *Antirrhinum*, suggesting that the functions of AS1, AS2, and ERECTA in specifying leaf adaxial identity are important in leaf adax-

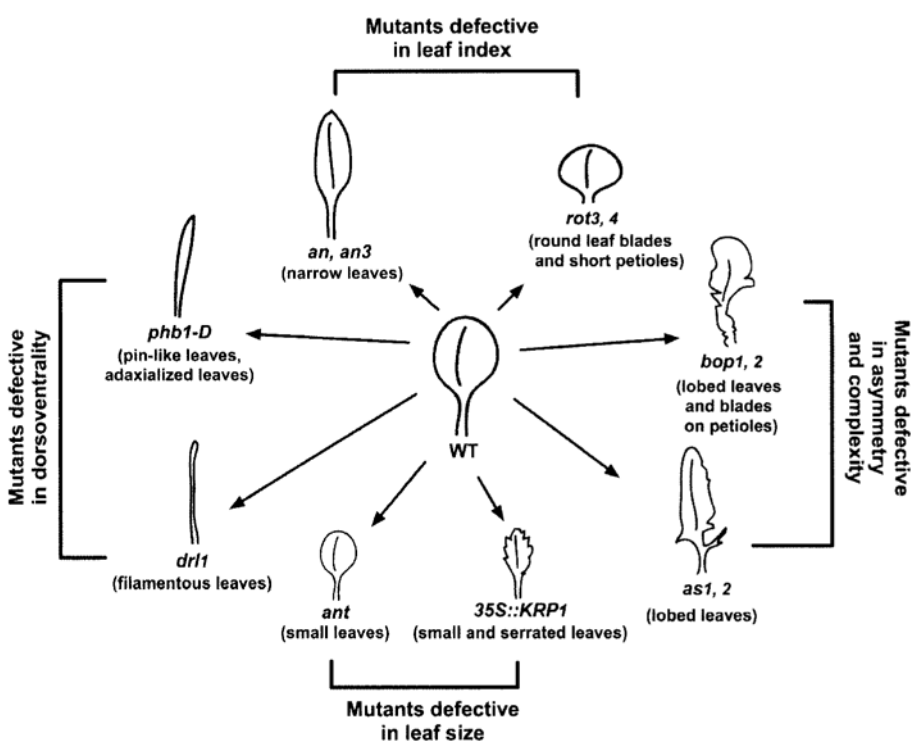

Figure 2. Representative morphology of leaves from *Arabidopsis* mutants deficient in dorsoventrality, symmetry, proximal–distal polarity, leaf index, and leaf size.

ial–abaxial polarity (Xu et al., 2003; Qi et al., 2004).

Nelissen et al. (2003) have reported that a mutation in the *DEFORMED ROOTS AND LEAVES1* (*DRL1*) gene, which encodes the ATP/GTP binding protein and is homologous with yeast TOT4/KTI12 associated with Elongator, shows narrow abaxialized leaves. Our recent molecular study has revealed that *DRL1* plays important roles in the adaxial–abaxial polarity of leaves by regulating SAM activity and the differentiation of leaf cells (unpublished data). Further elucidation of the mechanisms underlying processes in the early stages of leaf development will allow us to understand the role of temporal and spatial coordination of differential growth, as well as the link with hormonal regulation, during the formation of adaxial–abaxial polarity, flat morphology, and symmetry. Other recent reviews have discussed early events regarding flat morphology and symmetry in leaf development (Hay et al., 2004; Carraro et al., 2006; Kim and Cho, 2006; Tsukaya, 2006).

Regulation of cell division in leaf expansion

The close cooperation between cell proliferation and cell differentiation at each stage of development is an important factor affecting leaf morphogenesis (Fig. 3). Cell elongation continues throughout expansion, and division can occur during a limited period in immature leaves (Dale, 1976). Our previous genetic and histological research of the *curly leaf* (*clf*) mutation (Kim et al., 1998a) and a study of transgenic *Arabidopsis* harboring a G2/M-specific marker gene, CycB1 promoter-*GUS* (Donnelly et al., 1999), have revealed that the balance between leaf cell proliferation and enlargement, both spatially and temporally, is essential for the proper development of wild-type plants.

Factors affecting cell division to generate final leaf architecture can be divided into two classes: 1) general factors that control cell-cycle timing and pattern during the whole growth stage, e.g., the cyclin family (Cyc), cyclin-dependent kinases (CDKs), and inhibitors of CDKs (ICKs/ KRPs); and 2) regulatory factors that control cell proliferation directly or indirectly at certain stages and in a tissue-specific manner, e.g., *AINTEGUMENTA* (*ANT*) and *ARGOS* (Table 1). Inhibition of the cell cycle usually results in overall smaller leaves that contain fewer but larger cells (Hemerly et al., 1995), suggesting that altered leaf shape is caused by a reduction in the number of cells in the SAM.

A plant D-type cyclin, *CYCD3;1*, is expressed in specifically proliferating tissues such as meristems and developing leaves, but not in differentiated tissues. Plants over-expressing *CYCD3;1* show smaller but more cells in the merismatic regions and slower emergence of leaves than do wild-type plants. Likewise, the leaf shape in overexpressing plants is radically altered when distinct spongy and palisade mesophyll layers fail to develop (Dewitte et al., 2003). These results indicate that *CYCD3;1* promotes the cell cycle in the shoot apex and positively controls mitotic cell-cycle exit and differentiation in the leaves and vascular tissues (Dewitte et al., 2003).

Moreover, several molecular genetics studies of two cell proliferation inhibitors -- *ICK1* (cyclin-dependent kinase inhibitor 1) and *KRP2* (kip-related protein 2) -- support the idea that cell division directly affects leaf morphogenesis (Wang et al., 2000; Verkest et al., 2005). Plants overexpressing KRPs have fewer and smaller cells as a result of inhibited cell division and leaf expansion rates, but without a change in their temporal pattern of development, leading to significantly smaller and serrated leaves (Fig. 2; Wang et

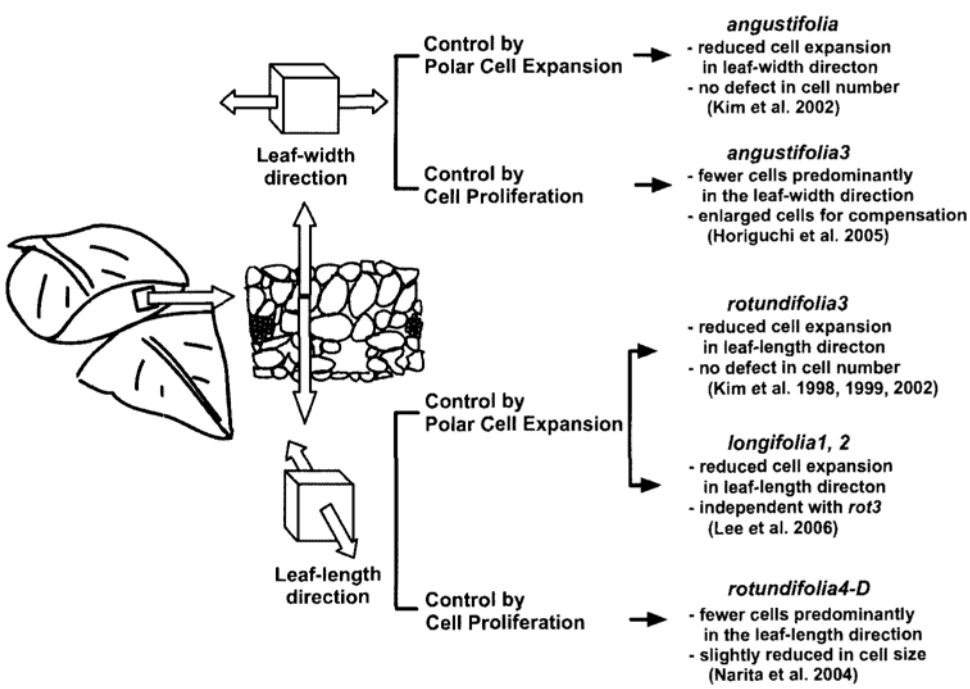

Figure 3. Polar control of cell proliferation and expansion in late leaf development. *ANGUSTIFOLIA* (*AN*) and *AN3* control width, whereas *ROTUNDIFOLIA3* (*ROT3*), *ROT4*, and *LONGIFOLIA* (*LNG*) control length during expansion. Despite similar external phenotypes, leaf cells from *an* or *an3* have defect in polar expansion or show uneven proliferation along width, respectively; leaf cells from *rot3, lng1 lng2*, and *rot4-1D* are defective in polar expansion and polar proliferation along leaf length, respectively.

al., 2000).

In addition to influencing the cell cycle itself, other factors that directly or indirectly control cell proliferation are important in determining leaf shape. *ANT*, which encodes a transcription factor with an AP2 domain in *Arabidopsis*, regulates the number of cells incorporated into developing leaves (Mizukami and Fischer, 2000). Loss of *ANT* functioning reduces the width and length of mature leaves throughout shoot development because of diminished cell division, leading to smaller leaves with fewer but larger-than-normal cells. In contrast, the overexpression of *ANT* results in larger leaves by increasing the number of cells without altering the external morphology. Therefore, *ANT* appears to prolong the period during which cells maintain their meristematic competence during organogenesis, without disrupting pattern controls (Mizukami and Fischer, 2000).

In examining the auxin-inducible *ARGOS*, Hu et al. (2003) have found that this gene is involved in determining lateral leaf size by regulating cell proliferation. Like those of *ANT*, the loss or gain of function of *ARGOS* causes decreases or increases in leaf size, except in the cotyledons, by modifying the duration of cell proliferation. Prolonged expression of *CycD3;1* has been detected in 35S:*ARGOS* leaves, similar to that in 35S:*ANT* plants. This suggests that *ARGOS* acts upstream of *ANT* to regulate leaf size as a regulator of the duration of *ANT* and *CycD3;1* expression via auxin-signaling (Hu et al., 2003). In addition, data have suggested that several genes, such as *STRUWWELPETER* (*SWP*) and *JAGGED* (*JAG*), act as positive regulators of cell division and promote differentiation in later leaf development (Autran et al., 2002; Ohno et al., 2004). Loss-of-function testing with *PEAPOD (PPD)* has demonstrated that the *ppd* mutant has a larger leaf lamina and produces dome-shaped, rather than flat, leaves (White, 2006). This mutant has prolonged outgrowths of laminar tissue due to the extension capacity of the margin cells, whereas plants over-expressing *PPD* have smaller and flat leaves caused by a reduction in the duration of cell proliferation, suggesting that *PPD* has a negative function in promoting cell division in the leaf margin.

Taken together, these results suggest two separate cell-cycle arrest mechanisms in leaf development: a primary mechanism that determines the termination of general cell division in the primordium, and a secondary mechanism in which dispersed meristematic cell proliferation is blocked by PDD activity (White, 2006). Further study of the interactions between the *PPD* genes and those promoting the maintenance of cell proliferation during leaf development will lead to an understanding of the roles of cell proliferation in leaf morphogenesis.

Regulation of cell proliferation with polarity in leaf expansion processes

Two-dimensional control of the polarity of cell proliferation possibly plays an important role in leaf morphogenesis (Fig. 3). A study of *rotundifolia4* (*rot4-1D*) suggests that this proliferation controls leaf length in a polar manner (Narita et al., 2004). That mutant has short, rounded leaf blades and short petioles, similar to the *rot3* mutant (Fig. 3). In contrast to *ant* mutants, which have fewer but enlarged cells in their leaves, *rot4-1D* has fewer cells, predominantly along the leaf length, but with normal cell sizes (Narita et al., 2004). This implies that ROT4 specifically controls cell proliferation along the long axis.

In contrast to *rot4-1D* mutants, our recent study of the *an3* mutant suggests that cell proliferation can control leaf width in a polar manner (Horiguchi et al., 2005). This mutant has narrow leaves, similar to the *ant* mutant (Fig. 3). Interestingly, that phenotype of *an3* is caused by an uneven reduction in the number of cells along the leaf axes, i.e., fewer, but larger cells, predominantly along the leaf width. *AN3* encodes a homolog of the human transcription coactivator that belongs to a small family of genes in the *Arabidopsis* genome (Horiguchi et al., 2005) and is identical to *GRF-INTERACTING FACTOR1* (Kim and Kende, 2004). In addition, AN3 interacts with AtGRFs, which have zinc-finger-like domains, in the yeast two-hybrid system. AtGRFs are proposed to play a role in cell proliferation in the leaf primordium (Kim and Kende, 2004). These data also indicate that AtGRF5 and AN3 interact and participate in the positive control of such proliferation, especially in the promotion of lateral leaf blade expansion.

Regulation of polar cell expansion in leaf expansion

As described above, the early phase of cell enlargement during leaf morphogenesis is closely related to the coordination of proliferation and elongation (Kim et al., 1998a). The late phase of enlargement involves a polar-dependent process of cell expansion. Our previous genetics studies of the *an* and *rot3* mutations have revealed that genetic regulation of the polar expansion of cells controls two-dimensional growth of the leaf blade (Fig. 3; Tsuge et al., 1996; Kim et al., 1998b, 1999, 2002). Genetic analysis has demonstrated that the *AN* gene regulates the width of leaf cells in a polarity-dependent manner and functions independently of *ROT3* (Tsuge et al., 1996). The *an* mutant has narrow leaves of normal length due to a defect in cell expansion in the leaf-width direction (Fig. 2), whereas the total number of leaf cells does not differ from that in the wild type. The *AN* gene encodes a member of the C-terminal binding protein (CtBP) family, all of which act as transcriptional co-repressors in animals. Its mutant shows an abnormal arrangement of cortical microtubules (MTs) in leaf cells, which are important in regulating polar elongation (Kim et al., 2002). These determinations support the idea that the *AN* gene controls the polarity of cell growth by influencing the arrangement of cortical MTs. Furthermore, microarray analysis suggests that *AN* regulates the expression of *MERI5*, a member of the xyloglucan endotransglucosylase family, in cell wall formation (Kim et al., 2002). These data imply that the *AN* gene modulates the polarity of cell expansion by controlling the arrangement of cortical MTs and/or cell wall formation.

In contrast to *an* mutants, *rot3* mutants have short leaves and petioles with normal leaf widths (Fig. 2), suggesting that *ROT3* specifically controls leaf length. The *rot3* mutant has a defect in its polar expansion of cells in the longitudinal direction, but its proliferation is normal (Fig. 2). A molecular examination of *ROT3* shows that it encodes a novel cyto-

chrome P450, CYP90C1 (Kim et al., 1998b), which is functional in brassinosteroid (BR) biosynthesis (Kim et al., 2005). In addition, *CYP90D1*, which is highly homologous to *ROT3* and involved in different steps of the downstream pathway in BR biosynthesis, is proposed to play important roles in plant development (Kim et al., 2005). BRs are general growth factors that regulate both the division and elongation of cells in all plant organs (Azpiroz et al., 1998). Therefore, *ROT3* appears to have evolved to specifically regulate leaf expansion.

Interestingly, a recent study of *ARGOS-LIKE* (*ARL*), which shares some sequence homology with the *ARGOS* gene described above, has shown that *ARL* is not involved in cell proliferation, but in cell expansion processes (Hu et al., 2006). Alteration in the dimensions of leaves from *ARL* transgenic plants is due to changes in their cell size, rather than number, suggesting that ARL plays a role in cell expansion-dependent leaf growth. Ectopic expression of *ARL* in *brassinosteroid insensitive1* (*bri1-119*) mutants partially restores cell growth in cotyledons and leaves, implying that ARL acts downstream of BRI1 and partially mediates BR-related cell expansion signals during leaf growth (Hu et al., 2006).

A new genetic component involved in the expansion of leaf cells in the leaf-length direction has been isolated by activation-tagging screens. A dominant mutant, *longifolia1-D* (*lng1-1D*), has elongated leaves and floral organs (Lee et al., 2006). These phenotypes are caused by the longitudinal expansion of cells in lateral organs. LNG1 is a novel protein in plants, but a BLAST search has identified several homologous proteins in *Arabidopsis* and rice with conserved regions (Lee et al., 2006). Because of redundancy, none of the single mutants of *lng1* and *lng2* differ from the wild type, but *lng1lng2* double mutants show decreased leaf expansion in the leaf-length direction (Lee et al., 2006). Similar to *ROT3*, these new factors, *LNG1* and *LNG2*, regulate the longitudinal expansion of cells (Fig. 3). Genetic analysis of *lng1-1D rot3-1* has suggested that LNGs and ROT3 act independently (Lee et al., 2006), but their molecular mechanisms are still unknown. Further biochemical and molecular studies of the LNG family members will increase our understanding of environmental controls in the polarity control of leaf expansion.

Taken together, all of our investigations have provided a blueprint for the genetic pathways for two-dimensional growth of leaf blades at the level of polar cell proliferation and expansion (Fig. 3).

CONCLUDING REMARKS

Since the mid-1990s, information about leaf formation has improved greatly. Recent research into early development has provided new evidence that both the appropriate temporal and spatial coordination for the activity of regulating factors and of hormones, e.g., auxin, GA, and CK, play key roles in the production of leaf primordia. Another important finding has been that transcription factors that integrate cell division with leaf development and the factors that control polarity-dependent expansion are essential for regulating final leaf shape and size. From a functional viewpoint, those two parameters are critical elements influencing proper adaptation to the environment. Unfortunately, however, our understanding of the molecular mechanisms for integrating these environmental components and of the inner developmental processes remains extremely limited. Further elucidation of the environmental controls that function during this development is essential to enhancing our knowledge of the control of leaf shape and size.

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