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Abrtract

The shoot system is the basic unit of development of seed plants and is composed of a leaf, a stem, and a lateral bud that differentiates into a lateral shoot. The most specialized organ in angiosperms, the flower, can be considered to be part of the same shoot system since floral organs, such as the sepal, petal, stamen, and carpel, are all modified leaves. Scales, bracts, and certain kinds of needle are also derived from leaves. Thus, an understanding of leaf development is critical to an understanding of shoot development. Moreover, leaves play important roles in photosynthesis, respiration and photoperception. Thus, a full understanding of leaves is directly related to a full understanding of seed plants.

The details of leaf development remain unclear. The difficulties encountered in studies of leaf development, in particular in dicotyledonous plants such as Arabidopsis thaliana (L.) Henyn., are derived from the complex process of leaf development, during which the division and elongation of cells occur at the same time and in the same region of the leaf primordium (Maksymowych, 1963; Poethig and Sussex, 1985). Thus, we cannot divide the entire process into unit processes in accordance with the tenets of classical anatomy.

Genetic approaches in Arabidopsis, a model plant (Meyerowitz and Pruitt, 1985), have provided a powerful tool for studies of mechanisms of leaf development in dicotyledonous plants, and various aspects of the mechanisms that control leaf development have been revealed in recent developmental and molecular genetic studies of Arabidopsis (for reviews, see Tsukaya, 1995 and 1998; Van Lijsebettens and Clarke, 1998; Sinha, 1999; Van Volkenburgh, 1999; Tsukaya, 2000; Byrne et al., 2001; Dengler and Kang, 2001; Dengler and Tsukaya, 2001; Tsukaya, 2001). In this review, we shall examine the information that is currently available about various mechanisms of leaf development in Arabidopsis. Vascular patterning is also an important factor in the determination of leaf shape, and this topic is reviewed in this resource by Turner (see also Dengler and Kang, 2001). The interested reader is also referred to work on the basic characterization of the vascular patterning in foliage leaves of Arabidopsis has been carried out by Candela et al. (1999) and Semiarti et al. (2001). For terminology, see Fig. 1.

MECHANISMS OF LEAF DEVELOPMENT

HISTORY OF STUDIES OF LEAF DEVELOPMENT IN ARABIDOPSIS

Many mutants of *Arabidopsis* were isolated with alterations in leaf morphology thirty and forty years ago (*e.g.,* Rédei, 1962; Lee-Chen and Steinitz-Sears, 1967; Barabas and Rédei, 1971), but each mutation was used merely as a positional marker for genetic mapping (*e.g.,* Koornneef *et al.,* 1983). The associated phenotypes were not analyzed initially in terms of developmental genetics, except in a few cases (Rüffer-Turner and Napp-Zinn, 1979). Early anatomical analyses of leaf development were reported for several other species, such as tobacco,

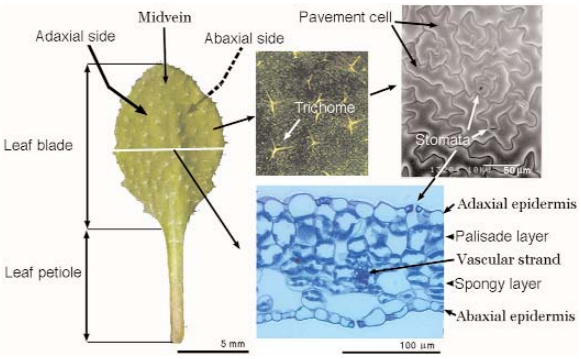

Figure 1. Terms used for description of leaf morphology. Left, gross morphology of the fifth rosette leaf of Arabidopsis. Upper right, magnified views of the leaf surface. Lower right, magnified view of cross section of the leaf blade.

Xanthium, and *Phaseolus* (for reviews, see Maksymowych, 1963; Marx, 1983; Dale, 1988) and, until the mid 1990s, the genetic and molecular analysis of leaf development was restricted to analysis of the function of the *knotted1* (*kn1*) gene of maize (*Zea mays* L.; Smith and Hake, 1992 and 1993). Apart from descriptions of heterophylly by Röbbelen (1957), studies of leaf development in *Arabidopsis* were very limited. Some morphological mutations in leaf shape were described (*e.g.,* Van Lijsebettens *et al.,* 1991), but isolation of such mutations was not a goal but, rather, a secondary result of attempts to of isolate new mutations.

The analysis of leaf development and its controls, using the techniques of developmental and molecular genetics, started a few years after the report of Pyke *et al*. (1991) of the kinetic and anatomic features of the development of the first set of foliage leaves of the Landsberg *erecta* strain of *Arabidopsis. Arabidopsis* leaves are very suitable material for studies of leaf morphogenesis because of their simple and stable form and the ease with which genetic analysis can be performed. 1994 marked a turning point in studies of leaf development, with *Arabidopsis* being recognized as a useful model plant. Tsukaya *et al.* (1994) performed an anatomical analysis of the development of cotyledons of the Columbia wild-type strain of *Arabidopsis* and showed that cotyledons could serve as a model system for studies of leaf morphogenesis. Moreover, they showed, for example, that the *angustifolia* (*an*) mutant has a defect in the polarity-dependent elongation of the cells in cotyledons. In the same year, Van Lijsebettens *et al.* (1994) revealed that insertion of T-DNA in the gene for the S18 ribosomal protein caused the *pointed first leaves* (*pfl*) phenotype, namely, extremely narrow first leaves and pale coloration at low temperatures. Lincoln *et al.* (1994) reported the isolation and characterization of the first homolog of the maize *knotted1* gene from the *Arabidopsis* genome, namely, the *KNAT1* (*knotted*-like from *Arabidopsis thaliana1*) gene.

Since the early 1990s, reports on new mutations in leaf morphology have been appearing with rapidly increasing frequency, for example, Jenks *et al.* (1996) isolated mutants with altered levels of epicuticular wax and leaf morphology (*knobhead* or *knb; bicentifolia* or *bcf; and wax*), Meisel *et al.* (1996) reported the *leaf morphogenesis7* (*lem7*) mutant which exhibits temperature-sensitive disorganization of the shoot apical meristem and leaf primordia, while Tsuge *et al.* (1996) reported the anatomical and genetic characterization of the *an* and newly isolated *rotundifolia* mutants. Tsuge *et al.* (1996) showed that the two-dimensional growth of leaf blades is controlled by two independent, polarity-dependent systems. They demonstrated that the *an* and *rot3* mutant phenotypes are specific to leaves and floral organs (modified leaves, as noted above), and theirs was the first developmental genetic analysis of mutations of the shape of *Arabidopsis* leaves.

Our understanding of leaf development in *Arabidopsis* has increased significantly since 1999 when Donnelly *et al.* (1999) analyzed the pattern of expression of a *cyc1At::GUS* fusion gene (with a destruction box inside the *GUS* coding region for b-glucuronidase). Expression of this gene is a specific marker of the G2/M phase of the cell cycle and Donnelly *et al.* were able to monitor the patterns of division and enlargement of leaf cells in leaf primordia from the first protrusion of leaf primordia to leaf maturity. In the same year, genetic categorizations of mutations in leaf morphology that had deposited in Stock Centers started to appear in the literature (Berná *et al.,* 1999; Serrano-Cartagena *et al.,* 1999 and 2000), as well as a description of the basic pattern of venation in leaf blades of *Arabidopsis* (Candela *et al.,* 1999).

With the progress of the *Arabidopsis* genome project, various genes for leaf development were isolated from *Arabidopsis.* For example, Kim *et al.* (1998) isolated the *ROT3* gene, which appears to control the polarity-dependent expansion of leaves in the leaf-length direction, utilizing strategy of T-DNA tagging and sequence data utilizing a strategy of *Arabidopsis* genome. Such cloning is becoming popular and easier. For example, McConnell *et al.* (2001) cloned the *PHB* and *PHV* genes, which have similar functions in the determination of the adaxial fate of leaf primordia, by searching for homologous genes shared by two chromosomal regions to which the two genes had been mapped. In parallel, a breakthrough occurred in studies of leaf development in *Arabidopsis,* with the identification of mutations (and genes) that affect the establishment of polarities around the leaf primordium and, in particular, dorsiventrality (*e.g.,* Bohmert *et al.,* 1998; McConnell and Barton, 1998; Lynn *et al.,* 1999; Sawa *et*

al., 1999; Siegfried *et al.,* 1999; Kerstetter *et al.,* 2001; McConnell *et al.,* 2001). Analysis of such mutations and genes has suggested that the establishment of polarities around the leaf primordium and the activity of shoot apical meristem might be tightly linked.

With the accumulation of information about the role of individual genes in the control of leaf development, the roles of genetic networks and of interactions among such genes have been the focus of much interest. As a result, many genes that affect leaf shape via the control of patterns of morphogenesis in the shoot apical meristem have been isolated. For example, certain genes were found to regulate the pattern of expression of *KNAT* genes around leaf primordia in *Arabidopsis* (*e.g.,* Byrne *et al.,* 2000).

Since leaf initiation is the most striking event that occurrs on the shoot apical meristem, studies of the genetic control of maintenance of the shoot apical meristem will certainly become more closely correlated with studies of leaf development. In the following sections, we shall summarize our present understanding of each process in the development of leaves in *Arabidopsis.*

EARLY EVENTS IN LEAF DEVELOPMENT IN ARABIDOPSIS

The early events in leaf development have been divided into three main processes (Foster, 1936, Steeves and Sussex, 1989, Smith and Hake, 1992), namely, the initiation of the leaf primordium, the establishment of dorsiventrality, and the development of a marginal meristem.

KNOX regulation around leaf primordia

Leaf initiation (**Fig. 2**) is the most important event in the morphogenesis of the shoot apical meristem (SAM) and the early events in leaf development and the activity of the SAM itself seem to be tightly linked. In *Arabidopsis*, genes such as *WUS, CLV1, CLV2, CLV3, KAPP,* and *STM* determine whether cells SAM are to remain stem cells or are to proceed along the pathway for organ formation, as reviewed elsewhere (Clark and Schifelbein, 1997; Fletcher *et al.,* 1998; Moussian *et al.,* 1998; Lenhard and Laux, 1999; Trotochaud *et al.,* 1999). Mutants with defects in genes for initiation of the SAM, such as the *shoot meristemless* (*stm*) mutant, lack a shoot meristem but develop leaves when regenerated from callus (Barton and Poethig, 1993). Thus, initiation of a SAM and of leaf primordia can be uncoupled. By contrast, the activity of the SAM has a major influence on the development of leaves. For example, in plants with a leaky allele of *stm, waldmeister* (*wam*), Felix *et al.* (1996) observed a number of defects in shoots, namely, altered leaf shape, in addition to occurrence of aerial rosettes, fasciation of inflorescence stems, disturbed gravitropism, and abnormal flowers.

Loss- and gain-of-function mutations indicate that *KNOX* (*Knotted-like homeobox*) genes, of which the *STM* gene is one (Long *et al.,* 1996), are important regulators of the function of the SAM. The *knotted1* (*kn1*) gene of maize encodes a homeobox gene (Vollbrecht *et al*., 1991) that is expressed in all SAM cells with the exception of the cells that will differentiate into lateral organs: leaf and floral organs (Smith *et al.,* 1992, Jackson *et al*., 1994). The dominant *Knotted1 (Kn1)* mutant of maize expresses *kn1* mRNA ectopically in leaf primordia and develops knot-like, meristematic tissues on its leaf blades. Analyses of molecular phylogeny, based on the sequences and patterns of expression of transcripts revealed that *KNOX* genes can be divided into two families in plants (Kerstetter *et al.,* 1994). Class I *KNOX* genes, which include *kn1*, are expressed in shoot apical meristems, and (with exceptions in species that have compound leaves) not in leaf primordia (**Fig. 3A**), while class II genes have more diverse patterns of expression. Species that develop compound leaves express class I *KNOX* genes in their leaf primordia with a few exceptions (Sinha, 1999).

Six *KNAT* genes have been identified in *Arabidopsis* (Lincoln *et al.,* 1994; Granger *et al.,* 1996; Serikawa *et al.,* 1996; 1997; Semiarti *et al.,* 2001; **Fig. 3B**). Ectopic expression of a Class I *KNOX* gene of *Arabidopsis*, *KNAT1*, in leaf primordia causes the formation of lobes and ectopic meristems in leaf blades (Lincoln *et al*., 1994; Chuck *et al.*, 1996). By contrast, ectopic expression of a Class II *KNOX* gene, *KNAT3*, does not induce severely lobed leaves (Serikawa and Zambryski, 1997; **Fig. 4**). Overexpression of another Class I *KNOX* gene, *STM*, results in a highly disorganized shoot apex with clusters of small, undeveloped leaf primordia (Williams, 1998). Thus, in *Arabidopsis*, expression of class I *KNOX* genes appears to be suppressed in leaf primordia during normal growth.

Many genes have been shown to control the patterns of expression of class I *KNOX* genes. The *PHANTASTICA* (*PHAN*) gene of snapdragon (*Antirrhinum majus* L.) encodes a MYB transcription factor and the product of the *PHAN* gene appears to suppress expression of *KNOX* genes in leaf primordia (Waites *et al.,* 1998). Loss of function of the *PHAN* gene reduces the amount of dorsal tissue in leaves (Waites and Hudson, 1995). Similarly, the

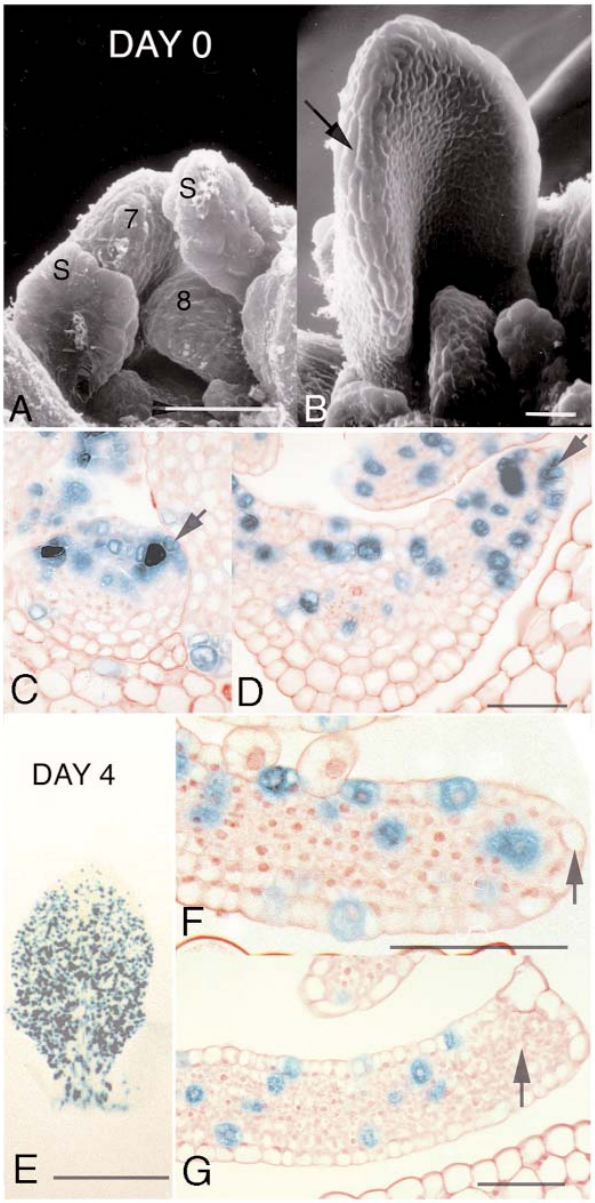

Figure 2. Early events in leaf development in *Arabidopsis.* Development of primordia of eighth foliage leaves (Leaf 8 primordia), as monitored with *cyclin1At::GUS* reporter gene that acts as a specific marker of the G2/M phase of the cell cycle. Blue color indicates the expression of the reporter gene. Arrows indicate cells at margin of leaf primordia. (A and B) Scanning electron micrographs of *gl1* leaves. Bar, 50 mm. (A) Leaf 8 primordium (indicated by 8) 50 mm in length. Also shown are Leaf 7 (7) and stipules (S) of older leaves. (B) Leaf 8 at stage of 0.4 mm in length. Arrow indicates enlarged cells at margin. (C) Cross section of Leaf 8 primordium at stage of Leaf 8 primordium (indicated by 8) 50 mm in length. (D) Cross section of Leaf 8 primordium at stage of 0.16 mm in length, sectioned at 25% above the base. Bar, 50 mm. (E) Cleared Leaf 8 at stage of 1.2 mm in length. Bar, 0.5 mm. (F and G) Cross sections of Leaf 8 primordia at stage of 1.5 mm in length. Bar, 50 mm. (F) Section taken 25% above leaf base. (G) Section taken 50% above leaf base. Modified from Donnelley *et al.* (1998; *Dev. Biol.* **215**, 407-419) with permission by authors.

rough sheath2 (*rs2*) gene of maize, a homolog of *PHAN*, prevents the accumulation of *kn1* mRNA in leaf primordia (Tsiantis *et al.,* 1999). In *Arabidopsis,* the *ASYMMETRIC LEAVES* (*AS*) gene is a homolog of the *PHAN* and *rs2* genes and the product of the *AS1* gene is expressed in the subepidermal cells of leaves and in the SAM (Byrne *et al.,* 2000). A loss-of-function type of *as1* mutation is associated with various morphological effects, such as crinkled asymmetric leaves (Barabas and Rédei, 1971; Tsukaya and Uchimiya, 1997), abnormally arranged leaf veins (Byrne *et al.,* 2000), decreased numbers of hydathodes and serrations in the leaf lamina (Tsukaya and Uchimiya, 1997; **Fig. 5**), and the occurrence of multiple midveins on leaves (Byrne *et al.,* 2000). The *AS1* gene acts negatively to regulate the class 1 *KNOX* genes of *Arabidopsis,* namely, *KNAT1* and *KNAT2* (Byrne *et al.,* 2000; Semiarti *et al.*, 2001). Expression of the *AS1* gene is, moreover, negatively regulated by the *SHOOT MERISTEMLESS* gene in the SAM (Ori *et al.,* 2000; Byrne *et al.,* 2000).

The pattern of expression of class I *KNOX* genes in the SAM is regulated not only by the *AS1* gene. Leaves of *as2* mutant plants resemble those of the *as1* mutant in shape (Tsukaya and Uchimiya, 1997; Ori *et al.,* 2000; Semiarti *et al.,* 2001; **Fig. 5**), and *as2* plants also express class I *KNOX* genes ectopically in their leaves (Ori *et al.,* 2000; Semiarti *et al.,* 2001). Analysis by reverse-transcriptase-PCR (RT-PCR) showed that *as2* plants express *KNAT1, KNAT2* and *KNAT6* genes in their young leaves, whereas the wild type does not express these *KNOX* genes at all (Semiarti *et al.,* 2001; **Fig. 6**). Ori *et al.* (2000) identified other factors that are involved in regulation of the expression of class I *KNOX* genes. They found, for example, that the *serrata* (*se*) mutation enhances phenotypes of *as1* and *as2* plants. Moreover, the phenotypes of *se as1 /as2* double mutants resemble that of transgenic *Arabidopsis* that overexpresses the *KNAT1* gene, with ectopic meristems on the sinuses of leaf blades (Ori *et al.,* 2000). The *SE* gene encodes a zinc-finger protein whose mRNA is expressed

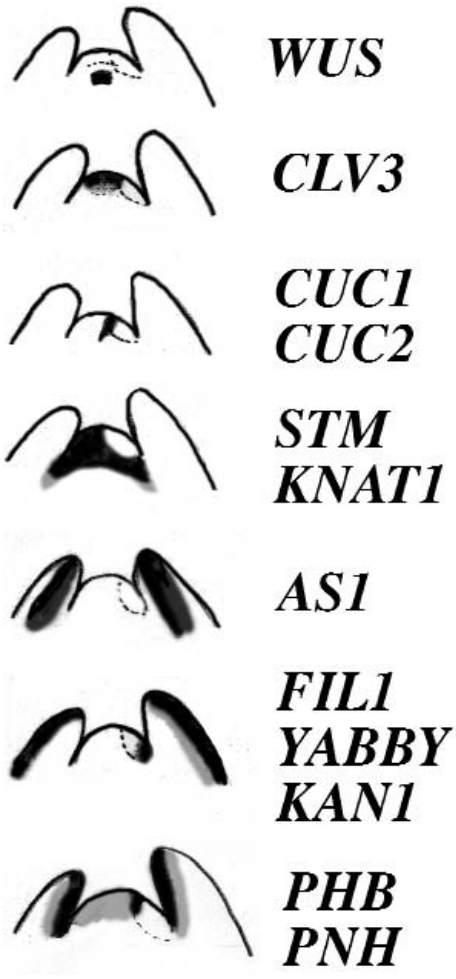

Figure 3A. *KNOX* genes in *Arabidopsis.*
Schematic representation of patterns of expression of genes required for early steps in leaf development. Cross sections of a shoot apex with two young leaf primordia and one predicted area of a leaf primordium are shown. Regions in which the indicated genes are expressed are shaded. See text for details.

in SAM and adaxial region of leaf primordia (Prigge and Wagner, 2001). Both *KNAT1::GUS* and *KNAT2::GUS* reporter genes were ectopically expressed in the sinuses of leaves of *as1, as2, as1 se,* and *as2 se* mutant plants (Ori *et al.,* 2000). However, the patterns of expression of *KNOX* genes in the SAM were not disturbed by these mutations, as indicated by the results of *in situ* hybridization; expression was restricted to the peripheral zone of the SAM and no signals were detected in leaf primordia (Ori *et al.,* 2000; Semiarti *et al.,* 2001). However, the level the *KNAT1* transcript in the basal part of leaf primordia was higher in *as1* and *as2* than in wild-type plants (Byrne *et al.,* 2000; Semiarti *et al.,* 2001). The *pickel* (*pkl*) mutation, which results in a defect in regulation of the primordia and SAM, has also been reported to enhance the *as* phenotype (Ori *et al.,* 2000). Regulation of the expression of *KNOX* genes around leaf primordia is probably quite complex and strictly controlled by a large number of different factors.

The role of the tightly regulated class I *KNOX* genes in the SAM remains an open question. The morphological phenotypes of plants that express Class I *KNOX* genes in their leaf primordia appear to be due to the abnormally prolonged proliferation of leaf cells in the lamina. Semiarti *et al.* (2001) reported that mature leaves of *as1* and *as2* plants retain the capacity for regeneration of shoots in culture on hormone-free medium. It was reported that levels of the transcripts of the *STM* and *KNAT1* genes were higher than in the wild type in transgenic *Arabidopsis* that overproduced cytokinin, which stimulated cell division (Rupp *et al.,* 1999). By contrast, studies of a tobacco *KNOX* gene, *NTH15* (Sakamoto *et al.,* 2001), revealed that the KTH15 protein directly suppresses the expression of a gibberellin biosynthetic (GA-biosynthetic) gene for GA 20-oxidase (*Ntc12*) and also reduces levels of bioactive GA. More detailed analysis of the functions of *KNOX* genes in the control of meristematic activity is needed if we are fully to understand the mechanisms of whereby these genes control leaf shape.

Establishment of leaf polarity

Mechanisms responsible for the establishment of proximo-distal and dorsiventral (dorsoventral) polarity in leaves of angiosperms have recently become 'hot topics' (*e.g.,* Hareven *et al*., 1996; Bohmert *et al*., 1998; Waites *et al.,* 1998; Sawa *et al.,* 1999; Siegfried *et al.,* 1999; Kerstetter *et al.,* 2001; McConnell *et al.,* 2001). The cited studies suggest that regulation of polarities along leaf primordia are closely related to the activity of the SAM.

Adaxialization

The morphological features of the abaxial sides of leaves are represented by the shape of abaxial epidermal cells, differentiation of the spongy layer, and the dorsiventral arrangement of xylem and phloem. The *phabulosa-1d*

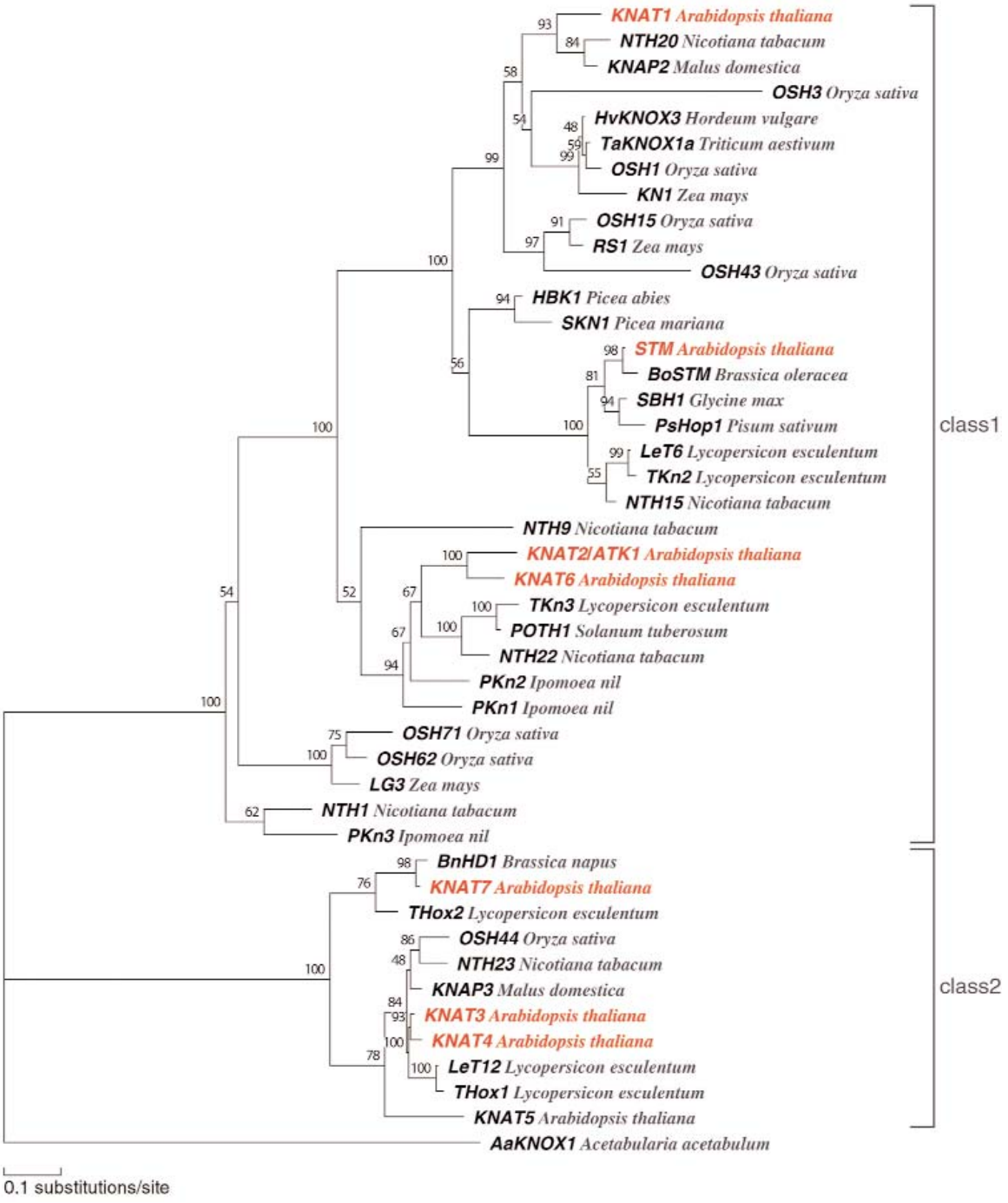

Figure 3B. *KNOX* genes in *Arabidopsis.*
Molecular phylogeny of *KNOX* genes. The maximum-likelihood tree of the KNOX genes found by local rearrangement search, rooted with *AaKNOX1*. Local boot strap probability by the resampling of estimating log likelihood (RELL) method is shown on branches. The horizontal branch length is proportional to the estimated number of amino acid substitutions per residue (bar = 0.1 amino acid substitution per residue). The brackets on the right indicate the classes of *KNOX* gene family. *KNOX* genes from *Arabidopsis* are shown in red color. Courtesy of Ms. Keiko Sakakibara (Speciation Mechanisms 2, National Institute for Basic Biology, Japan).

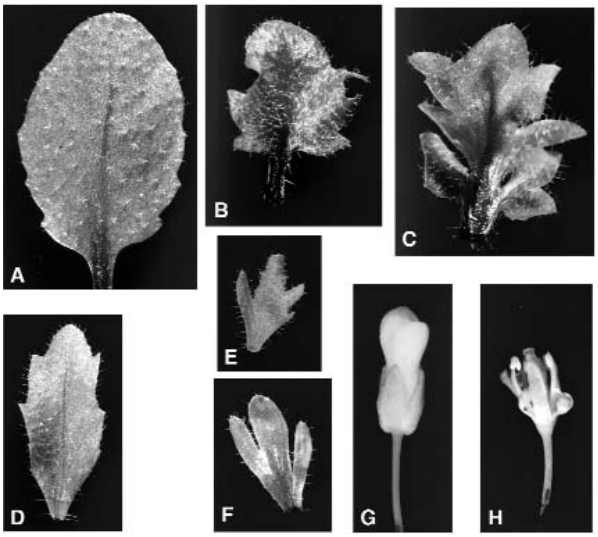

Figure 4. Phenotypes caused by ectopic expression of *KNAT1* gene in leaf primordia. The phenotype has been divided into two categories: type A is found in plants ectopically expressing a class I *KNOX, KNAT1;* and type B, which is similar to but quantitatively different from type A, and is found in plants ectopically expressing modified class II *KNOX, KNAT3* gene from that ELK-domain, homeodomain and C-terminus are removed and swapped with those of the *KNAT1.* The seventh rosette leaves of (A) wild-type, (B) type (B) and type A (C) plants are shown. The corresponding lowest cauline leaves are shown in (D), (E), and (F). (G) represents a wild-type or type B flower while (H) shows a type (A) flower. Photographs are reproduced from Serikawa and Zambryski (1997; Plant J. **11,** 863-869) with permission.

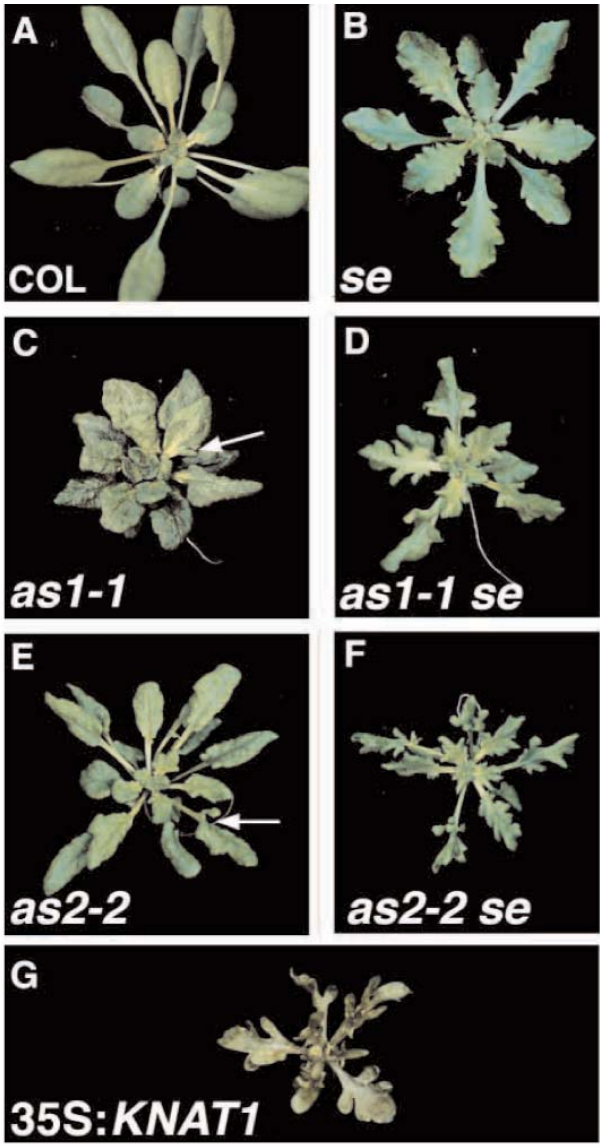

Figure 5. Gross morphology of rosette leaves affected by mutations of genes for regulation of patterns of expression of *KNOX* genes. Wild-type Columbia (A), single and double mutant plants (B – F), and transgenic *Arabidopsis* harboring 35S::*KNAT1* (G) are shown. Lobes are indicated by arrow in *as1* and *as2* leaves (C, E). Photographs are reproduced from Ori *et al.* (2000; Development **127,** 5523-5532).

(*phb-1d*; McConnell and Barton, 1998) mutation causes the adaxialization of leaves and all the abovementioned morphological features of the abaxial sides of leaves are absent from the strongly adaxialized leaves of the *phb-1d* mutant. Strongly adaxialized leaves are rod-like, while weakly affected leaves are trumpet-shaped (McConnell and Barton, 1998; **Fig. 7**). The inner surface of trumpet-shaped leaves was shown to be positive for expression of the *YABBY* gene, which encodes one of the factors involved in the abaxialization of cell fate, as discussed below (Siegfried *et al.,* 1999). The *phb-1d* mutant develops axillary SAMs, not only on the adaxial base of leaf, as does the wild type, but also on the abaxial base of leaf (McConnell and Barton, 1998). Thus, McConnell and Barton (1998) proposed that adaxial, basal leaf fate is required for the development of an axillary SAM and is also sufficient for direction of the formation of an axillary SAM. The *PHB* gene encodes a homeodomain-leucine zipper (HD-ZIP) protein, and was revealed to be *ATHB14* (McConnell *et al.,* 2001). The *phavoluta* (*phv*) mutation, which is associated with a very similar phenotype to that of *phb* plants, was isolated and found to be a mutation in another HD-ZIP (*ATHB9;* McConnell *et al.,* 2001). The

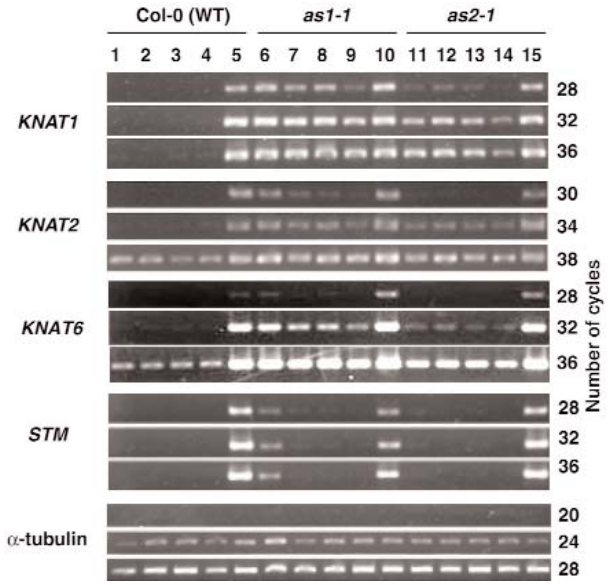

Figure 6. Analysis of transcripts of the *KNAT1, KNAT2, KNAT6* and *STM* genes in wild-type (WT), *as1-1* and *as2-1* shoot apices and rosette leaves by RT-PCR. The number of PCR cycles is indicated at the right of each panel. Lanes 1, 6 and 11, the first and second rosette leaves; lanes 2, 7 and 12, the third and fourth rosette leaves; lanes 3, 8 and 13, the fifth and sixth rosette leaves that had already expanded; lanes 4, 9 and 14, young leaves that had not yet expanded; lanes 6 – 10, *as1-1;* lanes 11 – 15, *as2-1.* Bottom panel shows products of control PCR that were amplified with primers specific for transcripts of the gene for a-tubulin. Reproduced from Semiarti *et al.* (2000; Development **128,** 1771-1783) with permission.

REVOLUTA/INTERFASCICULAR FIBERLESS gene, which plays an important role in the meristematic activity in the axillary SAM and leaf primordia (Talbert *et al.,* 1995), as well as in pattern formation of vascular tissue (Zhong *et al.,* 1997), is also a member of this gene family (Zhong and Ye, 1999; Ratcliffe *et al.,* 2000). Mutations in each of these three genes reduces the cell-proliferation activity in leaf blades and alters the pattern of formation of the axillary SAM.

The *PHB* transcript was found throughout the earliest stage of leaf primordium (P0), and its level increased in the following stages (P1 and P2), with localization to the adaxial domain of the leaf (McConnell *et al.,* 2001; **Fig. 3A**). Weak expression of the transcript was detected in the SAM region adjacent to leaf primordia at stages of P0 to P2, with highest levels at P0 and P1, suggesting that the adaxial domain of the leaf primordium and the SAM might behave as a unit. From a molecular analysis of the mutations in *phb* mutants and of the patterns of the expression of the *PHB* transcript, McConnell *et al.* (2001) proposed a model wherein the *phb-1d* mutation promotes the development of both the SAM and the adaxial region of a leaf primordium in parallel, as proposed by McConnell and Barton (1998). Since the mutations identified in *phb* and *phv* were confined to the region that encodes the START domain, which is thought to be the domain that regulates the activity of the gene product, McConnell *et al.* (2001) proposed that the PHB-activating ligand might unequally distributed in the leaf primordium, with highest levels in the adaxial domain and in the SAM region adjacent to the leaf primordium. The activated PHB might also positively control the synthesis or stability of its own transcript.

Shevell *et al.* (1994) reported that a mutation in a gene ,*emb30* (*gnom*), that resembles the gene for a yeast Sec7p-like protein caused abnormal dorsiventrality of cotyledons, with a palisade layer on the abaxial side and spongy layers on the adaxial side of the cotyledons. EMB30 might be involved in protein transport in secretory pathways, as is Sec7p in yeast. EMB30 might even be responsible for the unequal distribution of regulatory ligands, for example, for the abovementioned ligand for PHB.

The *pinhead/zwille (pnh*; Lynn *et al.,* 1999) mutant exhibits defects in leaf dorsiventrality similar to those of the *phb-1d* mutant, but the former exhibits a wider range of defects than the latter, namely, floral organs that are abnormal in both number and shape, embryos of aberrant shape, and abnormal embryogenesis (Lynn *et al.,* 1999). The *PNH* gene encodes a member of a family of proteins that includes the translation factor eIL2C (Lynn *et al.,* 1999). After embryogenesis, high levels of *PNH* transcripts can detected in developing vascular strands, with lower levels in the SAM and on the adaxial sides of leaves (Lynn *et al.,* 1999; **Fig. 3A**). This pattern is similar to that of the *PHB* transcript mentioned above (McConnell *et al.,* 2001). It has been proposed that the *PNH* gene encodes "a component of a meristem-forming competence factor" and might be involved in formation of the SAM. A double mutant both the *argonaute1 (ago1)* and *pnh* mutations did not express the SHOOT MERISTEMLESS protein in the SAM and the leaves failed to establish bilateral symmetry (Lynn *et al.,* 1999).

Abaxialization

Siegfried *et al.* (1999) reported that *YABBY* (*YAB*) genes, whose products are characterized by a zinc finger motif and a helix-loop-helix domain (Bowman and Smyth, 1999),

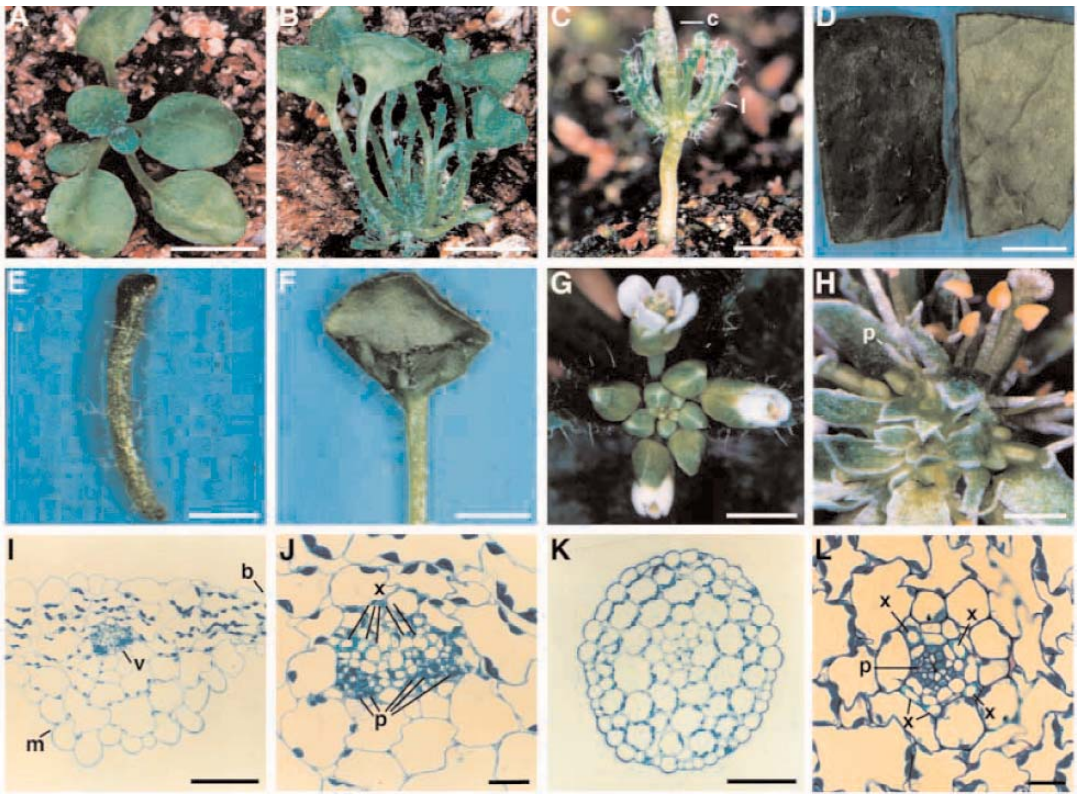

Figure 7. Leaves and floral organs of wild-type and *phb-1d* mutant of *Arabidopsis.* (A) Wild-type rosette. Bar, 5 mm. (B) Rosette of *phb-1d*/+ heterozygote. Note that leaves grow upward, with trumpet-like or rod-like shape. Bar, 5 mm. (C) Rosette of *phb-1d /phb-1d* homozygote. Foliage leaves (l) and cotyledons (c) are extremely radialized and grow vertically. Bar, 1.25 mm. (D) Adaxial (left) and abaxial (right) side of a wild-type foliage leaf. The adaxial surface is glossy, dark-green while the abaxial surface is matte, dull or pale green.Bar, 1.75 mm. (E) Severely adaxialized *phb-1d* leaf. The glossy, dark-green surface characteristic of the adaxial surface extends around the circumferunce of the radialized leaf. The petiole is highly reduced. Bar, 1 mm. (F) Less severely adaxialized leaf. This trumpet-shaped leaf exhibits adaxial characters on the outside of the cup. Inside of the cup shows abaxial characters. Bar, 1 mm. (G) Wild-type inflorescence. Bar, 2 mm. (H) Inflorescence of *phb-1d*/+ ; sepals fail to enclose the developing flower (p, petal). Bar, 1.25 mm. (I) Cross section of wild-type foliage leaf at midvein; adaxial surface is up (b, leaf blade; m, midrib; v, vascular tissue). Bar, 100 mm. (J) Close-up of wild-type vascular tissue of midrib (x, xylem; p. phloem). Bar, 20 mm. (K) Cross section of extremely radialized leaf of *phb-1d*/ + heterozygote. Bar, 100 mm. (L) Close-up of vascular tissue of a moderately radialized *phb-1d*/ + leaf. Note that xylem cells surround phloem cells. Bar, 20 mm. Photographs are reproduced from McConnell and Barton (1998; Development **125,** 2935-2942) with permission.

are involved in the abaxialization of lateral organs (leaves and floral organs) in *Arabidopsis.* The ectopic expression of a member of the *YAB* family, namely, *FILAMENTOUS FLOWER* (*FIL*) or *YABBY3* (*YAB3*) caused the ectopic differentiation of abaxial types of cell, while no acquisition of adaxial identity by leaves was apparent in the double null mutant, *fil yab3* (Siegfried *et al.,* 1999). *YAB* genes are expressed in the abaxial regions of lateral organs (Sawa *et al.,* 1999; Siegfried *et al.,* 1999). Thus, the *YAB* family appears to specify abaxial cell fate of leaves and floral organs. The *fil-5 yab3-1* double mutant occasionally developed epiphyllous shoots on the adaxial midribs of foliage leaves (Siegfried *et al.,* 1999; **Fig. 8**). Moreover, development of the SAM in overexpressors of a member of *YAB* was frequently arrested. In view of the role proposed for the *PHB* and *PNH* genes in the establishment of the dorsiventrality of leaves, there should be some relationship between maintenance of the SAM and the dorsiventrality

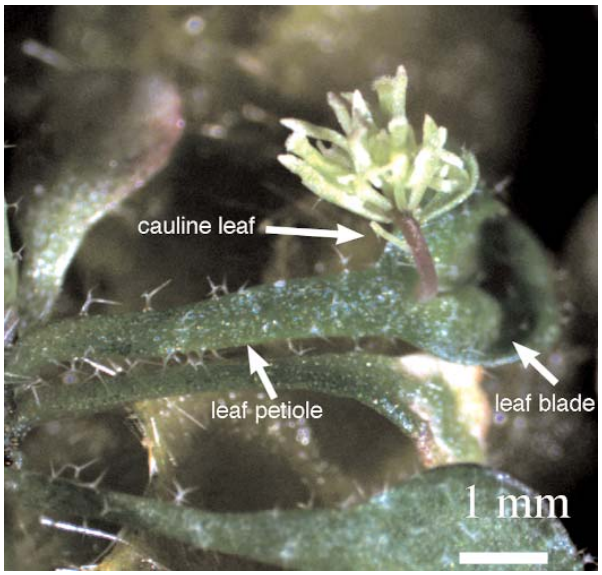

Figure 8. An epiphyllous inflorescence on the foliage leaves of the *yab3 fil* double mutant of *Arabidopsis.* Seeds were a kind gift from Dr. John Bowman (University of California at Davis, USA). Bar, 1 mm.

of lateral organs, as discussed elsewhere (Tsukaya, 2001). More detailed analysis of this possibility should provide clues to the genetic mechanisms that control establishment of dorsiventrality in leaves.

The *KANADI* (*KAN*) family of gene was also shown recently to regulate the abaxialization of leaf primordia (Kerstetter *et al.,* 2001). The *kan* mutation was originally identified as an enhancer of the abnormal dorsiventrality of carpels associated with the *crabs claw (crc)* mutation (Eshed *et al.,* 1999) and it was found to convert abaxial sides of leaves into adaxial sides (Kerstetter *et al.,* 2001). The *KAN* gene was cloned and found to encode a protein that includes a conserved GARP domain, which binds to a specific region of DNA. Moreover, a KAN-GUS fusion protein was found, appropriately, in nuclei when the transgene was expressed in onion epidermal cells (Kerstetter *et al.,* 2001). The *KAN* gene is expressed on abaxial sides of cotyledons in embryos and on the abaxial sides of leaf primordia and the primordia of floral organs. Moreover, transgenic plants that expressed *KAN* under control of the 35S promoter of cauliflower mosaic virus were found frequently to lack both a SAM and vascular tissue in the hypocotyl, having narrow, abaxialized cotyledons (Kerstetter *et al.,* 2001). As mentioned above, the pattern of expression of *PNH* is the mirror image of that of *KAN*, and the SAM and vascular tissue that the *KAN* transgenic plants lacked are defined by the expression of *PNH.* Thus, Kerstetter *et al.* (2001) proposed that "the specification of adaxial-abaxial polarity in lateral organs is intimately linked to the specification of central-peripheral identity in the shoot".

Occurrence of a marginal meristem

Conversion of the rod-shaped initial protrusions of leaf primordia into flat leaf primordia was believed at first to result from the activity of the meristem, which is established along the boundary of the abaxial and adaxial planes of the leaf primordium, namely, marginal meristem. Some researchers, such as Cusset (1986), questioned the potential importance of the marginal meristem in the morphogenesis of leaves. However, active cell division in marginal or submarginal regions of the leaf blade is thought to be necessary for the two-dimensional growth of leaves (Avery, 1933; Hara, 1957 and 1959). Recently, Donnelly *et al*. (1999) examined cell cycles in leaves of *A. thaliana* using, as a marker, a gene for b-glucuronidase (GUS) that was driven by the promoter of a gene from *Arabidopsis* for cyclin1 (*cyc1At*), which is expressed at the G2/M phase of the cell cycle. They found that the marginal meristem was quite active during the earliest phase of the development of the leaf primordium. However, the marginal meristem ceased to be active during the subsequent stages of development of the leaf primordium (**Fig. 2**) and the establishment of tissue layers and the expansion of regions of the leaf blade were not dependent on meristematic activity that was restricted to a particular zone. Proliferative cells in the primordia of leaf blades are distributed rather diffusely at the later stages of development. Moreover, Donnelly *et al*. (1999) showed that leaf development depends on the tissue-specific regulation of the cell cycle. These observations suggest that the plate meristem, which is situated deep inside the leaf primordium, might play an important role in morphogenesis of leaves (Donnelly *et al*., 1999).

The factors that induce or maintain meristematic activity in leaf primordia remained to be clarified. However, it seems plausible that there might be some link between activation of meristematic activity and the establishment of the boundary regions of the leaf primordium between two distinct positional values. In dicotyledonous plants, such as *Arabidopsis*, the marginal meristem might be induced between abaxial-adaxial zones(**Fig. 9**) established by the *YAB*, *KAN* and *PHB* genes. Formation of cambium, which is a meristematic tissue in stems, is also induced at the junction between two types of vascular tissue (xylem and

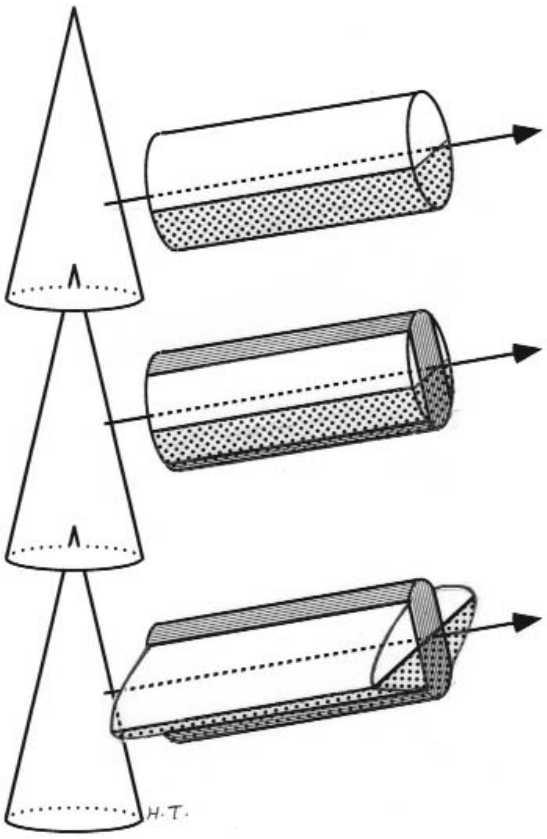

Figure 9. Dorsoventrality and polarity in the leaf-width direction in a leaf primordium.
A hypothetical process for the establishment of dorsoventrality and polarity in the leaf-width direction is shown schematically. Each cone indicates a shoot system. First, a leaf primordium (shown as a cylinder) establishes dorsoventrality (shown by light shading of the abaxial side of the primordium), which is based on a balance between its longitudinal polarity and the axial polarity of the shoot (upper third). Then a hypothetical zone of growth repression (shown by heavy shading) develops along the mid vein of the primordium (middle third). As a result, the leaf primordium starts to grow in the leaf-width direction exclusively, maintaining features of dorsoventrality (bottom third). Reproduced from Tsukaya (1998; J. Plant Res. **111,** 113-119) with permission.

phloem), and the polar arrangement of vascular tissue is regulated by the *REVOLUTA/INTERFASCICULAR FIBERLESS* gene, as mentioned above. It is norteworthy, in this context, that the polarity of leaf primordia and the polarity of vascular tissue are both controlled by HD-Zip proteins, as discussed above (Zhong and Ye, 1999; McConnell *et al.,* 2001).

GENETIC CONTROL OF LEAF EXPANSION IN ARABIDOPSIS

Leaf expansion is dependent on both the division and the elongation of cells. In dicotyledonous plants, the division and elongation of cells in the leaf blade occur without any obvious temporal and spatial patterns. For characterization of the unit processes that regulate the behavior of cells in leaf development, developmental genetic analysis seems to be the most effective strategy (Tsukaya, 1995). In this section, we shall summarize recent studies of the developmental genetics of leaf-expansion processes in *Arabidopsis*.

Polarity-dependent growth of leaf blades

The *angustifolia* (*an*) mutant of *Arabidopsis* (**Fig. 10**) was isolated as a mutant with narrower and thicker leaves than the wild type (Rédei, 1962). This phenotype was used initially as a visible marker for chromosome mapping, in the absence of complete anatomical analysis, as mentioned above. The phenotype of the *an* mutant is specific to leaves and floral organs (modified leaves), and it is caused not by a reduction in cell number but by a specific defect in the elongation of cells in the transverse (leaf-width) direction of the leaf (Tsukaya *et al*., 1994; Tsuge *et al*., 1996). This polar defect in cell elongation in the *an* mutant was observed in all leaf cells examined, including epidermal cells, trichomes, and parenchymatous cells of the leaf. The directional growth of parenchymatous cells is severely affected in the *an* mutant, with reduced expansion in the leaf-width direction and enhanced expansion in the leaf-thickness direction, as compared to the wild type. Thus, the *AN* gene is thought to be a key gene in regulation of the polar elongation of leaf cells in the leaf-width direction specifically (Tsuge *et al*., 1996).

Our interpretation of the *an* mutation led us to postulate the existence of another kind of mutant in leaf morphology, namely, a mutant with a polar defect in cell elongation in the longitudinal (leaf-length) direction of the leaf. Such a mutant was identified as the *rotundifolia3-1 (rot3-1)* mutant (Tsuge *et al*., 1996), which had a defect in the elongation of leaf cells in the leaf-length direction, without any change in the normal number of cells (**Fig. 10**). The phenotype was apparent only in leaves and floral organs. Thus, the *ROT3* gene appears to be the key gene that regulates the elongation of leaf cells in the leaf-length direction. The *an rot3-1* double mutant had an additive phenotype, suggesting that the two genes act independently (Tsuge *et*

al., 1996). The mutant phenotypes of *an* and *rot3-1* plants, in terms both of gross morphology and of the shape of cells in the leaf primordia, begins to appear at the stage of leaf development when the polarity-dependent expansion of cells begins.

Hanson *et al.* (2001) reported that high-level expression of the gene for a homeodomain leucine zipper (HD-Zip) transcription factor of *Arabidopsis, ATHB13,* resulted in narrow cotyledons that were very similar to those of the *an* mutant, when overexpressing seedlings were cultivated with high concentrations of sugars. Resembling the epidermal cells of *an* cotyledons (Tsukaya *et al.,* 1994), the epidermal cells of the narrow cotyledons of the overexpressor protruded less in the leaf-width direction and elongated more conspicuously in the leaf-length direction than wild-type epidermal cells. By contrast, the mesophyll cells of the overexpressor were normal in shape, size and number (Hanson *et al.,* 2001). The relationship between *AN* and *ATHB13* genes remains to be resolved.

The *ROT3* gene encodes a cytochrome P450 (CYP90C1; Kim *et al.,* 1998) with domains homologous to regions of steroid hydroxylases. Most homologous genes for cytochrome P450 in plants encode enzymes that are involved in the biosynthesis of brassinosteroid (Kim *et al.,* 1998; Kim and Tsukaya, 2001; see below for details). The pattern of expression of the *ROT3* gene does not exhibit any significant organ-specificity (Kim *et al.,* 1998). One allele, *rot3-2,* yielded slightly different phenotypes (**Fig. 10**) from the other null alleles and *rot3-2* plants had enlarged cells in their leaves and stems. The *rot3-2* allele encodes a mutation in an amino acid in a domain that might be involved in substrate recognition (Kim *et al.* 1998). Transgenic plants that overexpressed a wild-type *ROT3* gene had longer leaves than parent plants, without any changes in leaf width (Kim *et al.,* 1999; **Fig. 10**). The shapes of floral organs were also altered, but the elongation of stems, roots and hypocotyls was not severely affected. Thus, ROT3 appears to stimulate elongation of leaf cells specifically in the leaf-length direction. Transgenic plants that overexpressed the *rot3-2* gene had enlarged leaf blades but their leaf petioles were of normal length (Kim *et al.,* 1999; **Fig. 10**).

Other types of mutation that affect the polar expansion of leaves of *Arabidopsis* have been identified. Such mutant plants have leaves with defects only in the number of leaf cells along one specific axis. For example, the *an3* mutant, which is of this type, has narrow leaf blades of normal length (Tsukaya, unpublished data) and the *compact rosette (cro) 4-1* mutant has short leaves with leaf blades of slightly reduced width (Nakaya, Tsukaya, Murakami and Kato, submitted). These mutants have cells of normal size but reduced number in the lamina. Thus, both the size and the number of cells appear to be controlled in a polarity-dependent manner in the leaf lamina.

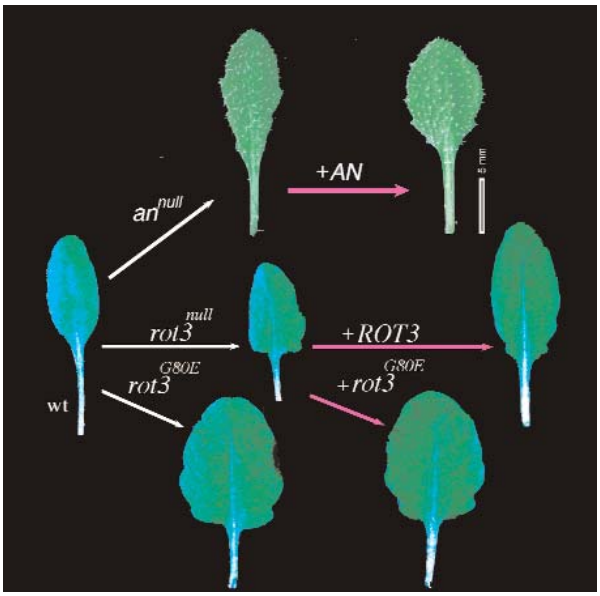

Figure 10. Genes that control the two-dimensional growth of leaves in *Arabidopsis.* The *AN* gene controls the width of the leaf lamina and the *ROT3* gene controls the lengths of leaf blades and petioles. Loss of function of these genes (indicated by 'null'; *an-1* in the case of *AN; rot3-1* in the case of *ROT3*) causes a decrease in the width or in the length of leaves, whereas overexpression of these genes (indicated by a plus sign) increases the respective dimension. A point mutation in *ROT3* that results in a particular change in the gene product (shown as G80E) increases the area of leaf blades, decreasing the leaf index and shortening of the length of the petiole. Bar, 5 mm.

Cortical microtubules and cell wall

The polarized expansion of plant cells is controlled by the orientation of cortical microtubules (MTs; for reviews, see Cyr, 1994; Shibaoka, 1994). The expansion of leaf cells is also controlled by the orientation of cortical MTs. For example, the *spiral2* (*spr2*) mutation shows the right-handed twisting of leaf petioles (Furutani *et al.,* 2000). Application of MT-interacting drugs changed the direction of the helical growth of the petioles from right-handed to left-handed and the effect is dose-dependent (Furutani *et al.,* 2000). Thus, right-handed twisting in leaf petioles in the *spr2* mutant is thought to be caused by a defect in the orientation of MTs (Furutani *et al.,* 2000).

Katanins are factors that cleave MTs and in a mutant, *fra2,* with a defect in the gene for a katanin-like protein (AtKTN1) of *Arabidopsis*, establishment of cortical MTs

after cytokinesis is delayed, with resultant defects in the shape of organs, in which cells are of reduced length but increased width (Burk *et al.,* 2001). Leaves of *fra2* plants have an altered leaf index (the ratio of leaf length/leaf width) as a result of a significant decrease in the lengths of leaf blades and leaf petioles and a slight decrease in the widths of leaves (Burk *et al.,* 2001).

The polarity of cells that is established by the cortical MTs is tranlated into the polarity of components of the cell wall, for example, the polarity of cellulose microfibrils. The loosening of the cell wall also influences the polarized expansion of each cell. Cho and Cosgrove (2000) manipulated the level of expression of expansin, a cell-wall-loosening protein, using antisense and sense sequences of the gene for an isoenzyme of expansin that is normally expressed in leaves, *AtEXP10.* The leaves of transgenic plants with an antisense gene had smaller leaf blades and shorter petioles and were often twisted, while transgenic leaves of overexpressors had slightly longer petioles and larger leaf blades than the wild type (Cho and Cosgrove, 2000).

Tsukaya *et al.* (1993) reported that *acaulis* (*acl*) mutants of *Arabidopsis* had extremely stunted inflorescences and extremely small leaves. Moreover, in *acl1* plants, the cells were significantly smaller than in the wild type both in leaves and in inflorescence stems. Thus, Tsukaya *et al.* (1993) proposed that the *ACL1* gene might be involved in the expansion of cells. Subsequently, Tsukaya *et al.* (1995) examined the possibility that *ACL* genes might function in the regulation of the arrangement of the cortical MTs and their experimental results eliminated this possibility. Cell expansion in leaves is affected by many factors. For example, the levels of the extremely small *fab2* mutant, in which cells are smaller than in wild-type leaves, contain significantly elevated levels of stearic acid (fatty acid, 18:0; Lightner *et al.,* 1994). A causal link between the morphological phenotype and the biochemical phenotype is suggested by the fact that a suppressor mutation, *shs,* substantially restored both a normal morphological and a normal biochemical phenotype (Lightner *et al.,* 1994). Thus, the *fab2* mutation appears to affect lipid composition, which, in turn, affects the expansion of cells and alters the size of leaves.

Hormonal controls

Although the actions of plant hormones are not specific to leaves, it is appropriate here to review the genetic controls of leaf shape that involve regulation of the perception or the biosynthesis of hormones in *Arabidopsis.*

Brassinosteroid

All known mutants of *Arabidopsis* with mutations related to the actions of brassinosteroid (BR) develop smaller leaves than the wild type. It is believed that the main role of BR is to stimulate the elongation of cells (Altmann, 1998). The *brassinolide insensitive 1 (bri1)* mutant (Li and Chory, 1997) has a defect in the perception of brassinosteroid and the *cabbage1 (cbb1 =dwf1-6; dim*), *cbb2*, *cbb3*, *constitutive photomorphogenesis and dwarfism* (*cpd*), *de-etiolated 2* (*det2*), *dwarf4* (*dwf4*), and *dwf5* mutants have defects in the biosynthesis of brassinosteroid (Feldmann *et al.,* 1989; Takahashi *et al.*, 1995; Kauschmann *et al.*, 1996; Szekeres *et al.*, 1996; Fujioka *et al.,* 1997; Azpiroz *et al.*, 1998; Cho *et al.,* 2000). Several steps in the biosynthesis of BR is catalyzed by cytochrome P450s, which are homologous to ROT3 in terms of amino acid sequence (for review, see Tsukaya and Kim, 2001). The *DET2* gene is thought to regulate the level of expression of the *KORRIGAN* (*KOR*) gene, which encodes a plasma-membrane-bound endo-1,4-b-D-glucanase that is essential for the initiation of cell expansion in *Arabidopsis* (Nicol *et al.,* 1998). The *kor* mutant develops smaller leaves than the wild type. Evidence is also beginning to accumulate to suggest that brassinosteroid might, in addition, be involved in the proliferation of cells (Hu *et al.,* 2000; Nakaya, Tsukaya, Murakami and Kato, submitted).

Auxin

Two types of histological defect in leaves have been reported in *auxin-resistant* (*axr*) mutants of *Arabidopsis.* The small leaves and short inflorescence stems of *axr1* plants, which have a mutation in a gene for ubiquitin-activating enzyme E1 (Leyser *et al.,* 1993), are attributed to decreases in the numbers of cells that make up these organs (Lincoln *et al.,* 1990). By contrast, in the *axr2* mutant, there is a dramatic decrease in the lengths of cells in stems, with a less conspicuous decrease in cell number. However, no anatomical studies of *axr2* leaves have been reported to date (Timpte *et al.,* 1992). The *AXR2* gene encodes a member of the Aux/IAA family that is thought to play a role in auxin signaling (Nagpal *et al.,* 2000). The *AXR1* gene might be involved in the signaling that is related to the auxin-dependent proliferation of cells and the *AXR2* gene might be involved in the expansion of cells.

Gibberellic acid and abscisic acid

Although many dwarf, small-leaved mutants of *Arabidopsis* have been shown to have defects in the biosynthesis or perception of gibberellic acid (GA; for reviews, see Hedden, 1999; Sun, 2000), the anatomical aspects of the mutant leaves have not been fully analyzed.

Abscisic acid (ABA) is known to control the closure of stomata on leaves but it is unclear whether ABA might be involved in regulation of the growth of leaves (for review, see Dale, 1988). Recently, GPA1, the a subunit of a prototypical heterotrimeric GTP-binding protein of *Arabidopsis,* was found to regulate ion channels and ABA signaling in the guard cells of leaves (Wang *et al.* 2001). Moreover, in *gpa1* mutant plants,the division of cells in leaves and stems was limited, and responsiveness to BR was reduced (Ullah *et al.* 2001). There might be cross-talk in leaf cells between BR and ABA and/or their signalling pathways.

Phytochrome-mediated control of leaf development

Developmental plasticity in response to environmental and physiological conditions is a unique feature of plant development and is one of the most important current targets of studies of mechanisms that control plant development (Sultan, 2000). Among the environmental factors that influence the developmental plasticity of plants, light has a particularly significant effect on leaf morphology, since leaves should receive photons as much as possible for photosynthesis in adaptation to the light environment.

The PHYTOCHROME (PHY) gene controls the expansion of leaf blades and the elongation of petioles in *Arabidopsis* (Goto *et al.,* 1991). Mutational analysis of *PHY* genes revealed the roles of individual phytochromes in *Arabidopsis,* as reviewed in this volume by Deng and Chory. The *far-red elongated1* (*fre1 = phyA)* mutant was reported to exhibit no changes in leaf morphology (Nagatani *et al.* 1993), while the long-hypocotyl *hy3* (= *phyB*) mutant does have defects in leaf morphology (**Fig. 11**). All studies of this mutation are in agreement that *hy3* mutant plants have longer petioles than those of wild-type plants (Goto *et al.,* 1991; Nagatani *et al.,* 1991; Robson *et al.,* 1993; Nagatani *et al*., 1993; Reed *et al.,* 1993). Moreover, the effect of the PHY B-mediated perception of light on the leaf blade appears to be much smaller than that on the leaf petiole (Tsukaya and Kim, unpublished

Figure 11. Gross morphology of rosette and flowering plants of the wild type (left) and the phyB-9 mutant (right). Plants were cultivated at 22°C under 12 hours of strong light and 12 hours of darkness dairy. Bar, 5 mm.

data). Recently, Lake *et al.* (2001) reported that signals perceived by old mature leaves, for example, light intensity and the concentration of CO_2, are transduced to newly developing leaf primordia and control the density of stomata on the surfaces of new leaves in *Arabidopsis*. Long-distance signaling among organs might be quite important for the regulation of leaf development, in particular, in the adaptation to changes in environmental factors.

Cell proliferation

The proliferation of leaf cells is affected by many factors but most such factors influence cell proliferation ubiquitously in all plant organs. Kim *et al.* (1998b) analyzed a mutant with a defect in the *CURLY LEAF* (*CLF*) gene, which encodes a member of the family of polycomb proteins that is required for stable repression, in vegetative shoots, of a member of *MADS* gene family, the *AGAMOUS* (*AG*) gene (Yanofsky *et al.,* 1990). The *clf* mutant develops normal roots, hypocotyls and cotyledons, but the foliage leaves are significantly smaller and the inflorescence stems are shorter than those of the wild type. Both the extent of cell elongation and the number of cells are reduced in the *clf* mutant (Kim *et al.,* 1998b). The *AG* gene not only acts in the identification of floral organs but also plays a role in preventing continued cell division in a particular region in the floral meristem (for review, see Meyerowitz, 1997). Thus, it is possible that ectopic expression of the *AG* gene in vegetative shoots, as a result of loss of function of the *CLF* gene, might act directly to arrest cell division in *clf* leaves. This idea is supported by the fact that ectopic expression of *AG* results in the small leaves, as *clf ag* double mutant have large leaves (Goodrich *et al.,* 1997). It remains to be determined why elongation of leaf cells is arrested in *clf* mutant plants in which the *AG* gene is ectopically expressed.

Mutations in ribosomal proteins sometimes result in pointed leaves (Van Lijsebettens *et al*., 1991 and 1994; Ito *et al*., 2000). As mentioned above, Van Lijsebettens *et al.* (1994) showed that insertion of T-DNA in the gene for an S18 ribosomal protein caused the *pointed first leaves* (*pfl*) phenotype, namely, pointed narrow first leaves and pale coloration, when plants were cultivated at low temperature. Ito *et al.* (2000) examined the anatomy of the leaves of a *pfl2* mutant of *Arabidopsis* with a disrupted form of the cytoplasmic ribosomal protein (RP) S13. They found that the leaves contained fewer and larger cells than the wild type. The narrow, pointed leaves of the *pfl* mutants might be due to retardation or cessation of the proliferation of leaf cells in the lamina, which might be caused, in turn, by a shortage of a necessary ribosomal protein. The increase in cell volume in *pfl2* leaves can be explained by the putative compensatory system that will be discussed below.

A compensatory system

As reviewed and discussed by Tsukaya (2001), it seems to be a general rule that if the progress of the cell cycle is retarded or ceases earlier than normal in a leaf primordium, as a result of the introduction of a transgene, the number of cells in the leaf lamina decreases and, at the same time, each leaf cell tends to grow larger than the wild-type cells. In *Arabidopsis*, a loss-of-function mutation of the *AINTEGUMENTA (ANT)* gene (Mizukami and Fischer, 2000; **Fig. 12**), overexpression of the gene for an inhibitor of cyclin-dependent kinase (ICK1; Wang *et al.*, 2000) and disruption of a GTP-binding protein, GPA1, which is thought to be a modulator of cell division (Ullah *et al.*, 2001) are all associated with such a phenomenon. The *pfl2* mutant mentioned above (Ito *et al.,* 2000) might also provide a similar example. In each case, leaves are of reduced size, as compared to wild-type leaves, the number of cells in the lamina decreases while the volume of leaf cells increases. There seems to be little doubt that a compensatory system must play a role in coordination of the behavior of cells in a lamina (Tsukaya, 2001), but the results do not necessarily support the ideas of Organismal theorists, who have proposed that "genetic information specifies leaf form independently of genetic influences on sizes and shapes of cells and on extents and orientations of cell divisions" (Kaplan and Hagemann, 1991).

Cell theory provides a more plausible explanation of the abovementioned results, since, to date, no evidence has been presented that a decrease in cell volume can accelerate in cell division or, conversely, that an increase in cell division can suppress the expansion of cells (for review, see Tsukaya, 2001). Leaf size was increased upon overexpression of the *ANT* gene, as a result of increases in numbers of cells, but the sizes of cells were unchanged (Mizukami and Fischer, 2000). Moreover, Cockcroft *et al.* (2000) reported that the D-type cyclin CycD2 increased cell division, with the development of larger leaves than normal. Thus, an increase in cell division does not seem to trigger suppression of the expansion of cells in the leaf lamina of *Arabidopsis*. The mechanism responsible for the compensatory system(s) might be very important for an understanding of leaf development. For discussions of the validity of Cell theory and a proposed Neo-Cell theory, the reader is referred to a recent review (Tsukaya, 2001).

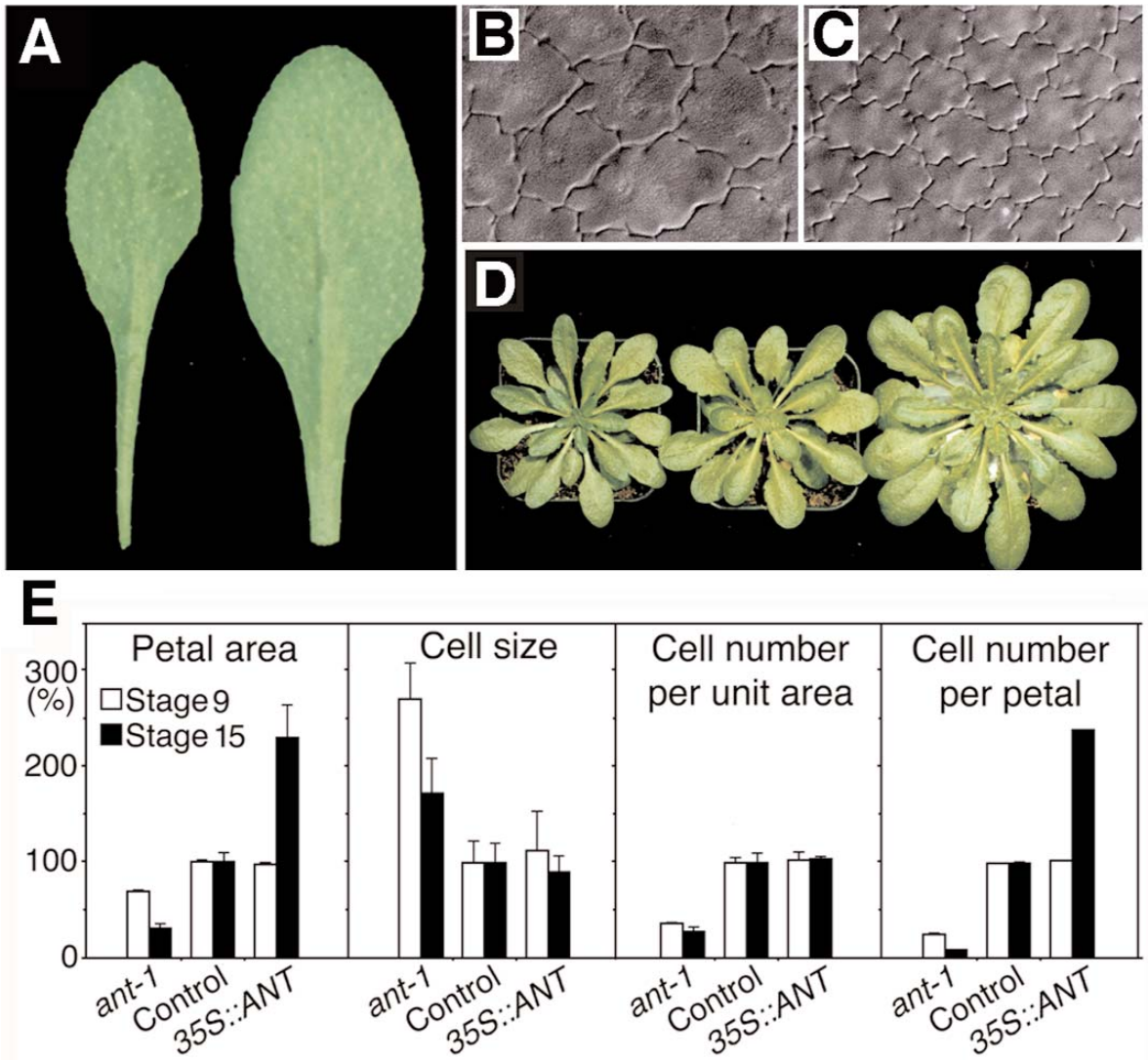

Figure 12. Loss of function and gain of function of the AINTEGUMENTA (ANT) gene cause alterations in the sizes of leaves and floral organs. (A) Fully grown seventh-foliage leaves from an ant-1 plant (left) and a wild-type plant (right). (B and C) Epidermal cells at the same magnification from the abaxial, distal portions of mature ant-1 (B) and wild-type (C) petals. (D) Gross morphology of ant-1 (left), control (middle), and 35S::ANT (right) plants grown under the same conditions (35S represents the 35S promoter of cauliflower mosaic virus). (E) Comparisons of petal area, cell size, cell number per unit area, and numbers of cells per petal. Results from ant-1 and 35S::ANT petals are shown as percentages of values from control petals. Modified from Mizukami and Fischer (2000; Proc. Natl. Acad. Sci. U.S.A. 97, 942-947) with permission.

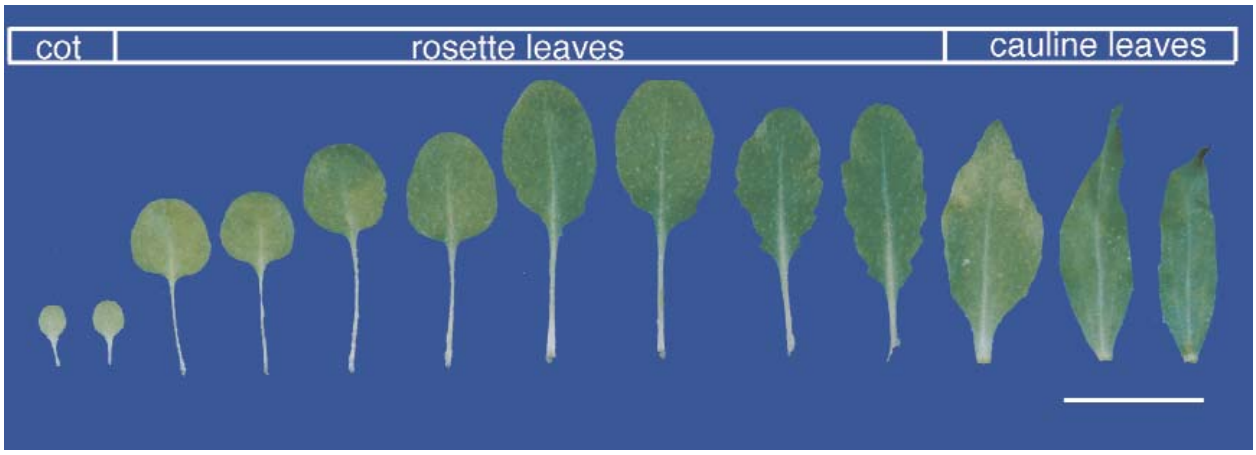

Figure 13. Heteroblasty in *Arabidopsis* (Columbia wild type) under continuous light, 22^{O} C. The photograph shows gradual changes in the shape of leaves. *From left*: two cotyledons (cot); eleven foliage, rosette leaves (rosette leaves); and three cauline leaves. Foliage leaves and cauline leaves are arranged from left as: first foliage leaf; second foliage leaf; third, fourth ... eleventh foliage leaf; first, second, and third cauline leaf. Bar = 5 mm. Reproduced from Tsukaya *et al.* (2000; Planta **210,** 536-542) with permission.

IDENTIFICATION OF LEAVES

Finally, let us briefly discuss two types of leaf-like structure, namely, the cotyledon and the leaf petiole. Cotyledons are leaf-like structures that develop during embryogenesis. The leaf petiole is usually regarded as the proximal part of the leaf. Let us examine these interpretations designations in terms of the results of mutational analysis.

Identity of cotyledon in terms of heteroblasty

Heteroblasty or heterophylly, namely, age-dependent changes in the morphology of foliage leaves, is a feature of the foliage leaves of most species. *Arabidopsis* exhibits typical heteroblasty and leaf shape changes from the juvenile, through the early adult, to the late adult phase (**Fig. 13**; Röbbelen, 1957; Tsuge *et al.,* 1996), with concomitant changes in the patterns of distribution of trichomes on leaf surfaces (Chien *et al.,* 1996; Telfer *et al.,* 1997). The cauline leaves lack petioles (Tsukaya, 1995; Tsuge *et al.*, 1996). The contours of the first and second true leaves resemble those of cotyledons but the leaves have trichomes and a complex vascular system. Thus, the cotyledons might be considered to be as a particular type of foliage leaf and it might be appropriate to examine the cotyledon in the context of heteroblasty.

In *Arabidopsis*, the number of cells in a single cotyledon is relatively small, and the synchronous culture of cotyledons is rather easy (Tsukaya *et al*., 1994). In addition, expansion of cotyledons after imbibition of seeds depends mostly on the expansion of cells. Thus, the role of cell expansion in the morphogenesis of cotyledons can be analyzed separately from the cell-division process and the cotyledon is, thus, a good model system for studies of leaf development. For example, the *angustifolia (an)* mutant of *Arabidopsis* has narrow cotyledons, and this phenotype was the first to shown to be due to a defect in the polar elongation of cells (Tsukaya *et al.,* 1994).

However, cotyledons are different from foliage leaves in so far as a cotyledon is not derived from the shoot apical meristem, from which all foliage leaves differentiate. This statement is supported by the fact that the *stm-1* mutant (Barton and Poethig, 1993) and the *wus* mutant (Laux *et al.,* 1996) of *Arabidopsis* develop cotyledons normally but do not differentiate a shoot apical meristem and a foliage leaves. Moreover, some mutants with alterations in leaf shape exhibit their mutant phenotypes in foliage leaves specifically and exclusively (Tsukaya *et al.*, 2000). However, cotyledons and true leaves do seem to share a common developmental background to some extent.

Conway and Poethig (1997) reported that the *extra cotyledon* (*xtc*) and *altered meristem programming1* (*amp1* = *pt*) mutations each transformed a few early foliage leaves

into cotyledon-like organs. Thus, cotyledons can, in fact, arise from the SAM. The *amp1* mutant was originally isolated as a mutant with an elevated level of cytokinin (Chaudhury *et al.,* 1993) and the *AMP1* gene appears to be intimately involved in control of the activity of the SAM (Rupp *et al.,* 1999). By contrast, in *leafy cotyledon (lec)* mutants (Meinke 1992), cotyledons have some features that resemble those of true leaves, such as absence of seed storage proteins, differentiation of trichomes, and absence of drought tolerance. The *lec1* mutation was thought, at first, to reflect the homeotic conversion of cotyledons into true leaves (Meinke, 1992) but the phenotype is now interpreted as a type of modification (West *et al*., 1994).

Tsukaya *et al.* (2000) analyzed heteroblastic traits in the *lec1* mutant and wild-type *Arabidopsis,* using a marker gene that indicated the number of hydathodes per leaf blade, which increases gradually (Tsukaya and Uchimiya, 1997), as a quantitative parameter of heteroblasty. The ectopic expression in *lec1* cotyledons of the developmental program for the first foliage leaves seemed also to affect the heteroblastic features of the first set of foliage leaves, while foliage leaves beyond the third position appeared normal. Similarly, in wild-type plants, discrepancies in heteroblastic features, relative to standard features, of foliage leaves at early positions seems to be eliminated in foliage leaves at later positions (Tsukaya *et al.,* 2000). Similar phenomena are associated with several mutations that affect regulation of the activity of the SAM (*e.g., wus*; Hamada *et al.,* 2000). These results suggest that heteroblasty in foliage leaves might be affected, in part ,by the heteroblastic stage of the preceding foliage leaves but is finally controlled autonomously at each leaf position and, furthermore, that cotyledons can indeed be considered in the context of heteroblasty.

The petiole and the stem

The proximal part of *Arabidopsis* leaves is axial and bladeless and is called the leaf petiole. In terms of differences between leaf petioles and leaf blades, three classes have been recognized with respect to mutations in leaf morphology that have been identified to date, namely, leaf-blade-specific mutations, petiole-specific mutations, and mutations that affect both the leaf blade and the petiole. In addition, some mutants with defects in stem elongation exhibit the same defects in of petiole elongation. As mentioned above, regulation by light of the elongation of leaf petioles is more significant than regulation by light of the expansion of leaf blades. For example, the *acaulis2* (*acl2*) mutant was first identified as a mutant with inflorescences of much reduced length (Tsukaya *et al.,* 1993) but Tsukaya *et al.* (1995) showed that the *acl2* mutant has a similar defect in the elongation of inflorescences and leaf petioles, which are both axial organs. As also discussed above, the phytochrome-mediated control of elongation is apparent in axial organs, such as hypocotyls and petioles (**Fig. 11**). Anatomical studies of the early phases of petiole development suggest that leaf petioles develop differently from leaf blades (Foster, 1936). Taken together, the results suggest that development of the petiole might be regulated differently from that of the leaf blade, at least to some extent.

Recently, van der Graaf *et al.* (2000) reported that activation of the *LEAFY PETIOLE* (*LEP*) gene, which encodes a protein with a domain similar to the DNA-binding domain of members of the AP2/EREBP family of transcription factors, converted the proximal part of the leaf from petiole into leaf blade. The *blade-like petiole* (*blp*) mutant has a similar phenotype, with leaflet-like structures on leaf petioles and the proximal parts of leaf blades (Ha *et al.,* 2001). Analysis of these mutations and genes might provide clues to the genetic identification of the petiole and the leaf blade.

CONCLUDING REMARKS AND PERSPECTIVES

Molecular mechanisms of morphogenesis in multicellular organisms are important targets of research in modern biology. As discussed at the beginning of this chapter, leaf morphogenesis is a process that is unique to plants and furthermore, the leaf is a critical organ for a full understanding of shoot morphogenesis in angiosperms. The introduction of the techniques of developmental genetics has provided many tantalizing hints, and future studies of leaves and modified leaves will complement each other and help us to understand shoot morphogenesis and also, as a consequence, plant morphogenesis.

Clarification of mechanisms of leaf development in *Arabidopsis* also allows studies of leaf development from the perspective of evolutionary, developmental biology (Evo-devo; Tsukaya, 1995b). In the near future, Evo-devo studies of leaf development will certainly proliferate. However, while studies of *Arabidopsis* leaves have advanced our understanding of the fundamental mechanisms that control leaf development, some aspects of leaf development, for example, the formation of compound leaves, required characterization of the genetic mechanisms of development of leaves of tomato, pea and

other well-studied species with compound leaves (Sinha, 1997; Goliber *et al.,* 1999). We must study not only the leaves of the model plant *Arabidopsis* but also the leaves of other species. However, information obtained from studies of *Arabidopsis* leaves will continue to provide an important foundation for such future studies.

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REFERENCES

Altmann, T. (1998). Recent advances in brassinosteroid molecular genetics. *Curr. Opin. Plant Biol.* **1**, 378-383.

Avery, G.S. (1933). Structure and development of the tobacco leaf. *Amer. J Bot*. **20**, 565-593.

Azpiroz, R., Wu, Y., LoCascio, J.C., and Feldmann, K.A. (1998). An Arabidopsis brassinosteroid-dependent mutant is blocked in cell elongation. *Plant Cell* **10**, 219-230.

Barabas, Z., and Rédei, G.P. (1971). Facilitation of crossing by the use of appropriate parental stocks. *Arabidopsis. Inf. Ser.* **8**, 7-8.

Barton, M.K., and Poethig, R.S. (1993). Formation of the shoot apical meristem in *Arabidopsis thaliana*: an analysis of development in the wild type and in the *shoot meristemsless* mutant. *Development* **119,** 823-831.

Berná, G., Robles, P., and Micl, J. L. (1999). A mutational analysis of leaf morphogenesis in *Arabidopsis thaliana. Genetics* **152**, 729-742.

Bohmert, K., Camus, I., Bellini, C., Bouchez, D., Caboche, M., and Benning, C. (1998). *AGO1* defines a novel locus of *Arabidopsis* controlling leaf development. *EMBO J.* **17**, 170-180**.**

Bowman, J.L., and Smyth, D.R. (1999). *CRABS CLAW,* a gene that regulates carpel and nectary development in *Arabidopsis,* encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* **126**, 2387-2396.

Burk, D.H., Liu, B., Zhong, R., Morrison, W.H., and Ye, Z.-H. (2001). Katanin-like protein regulates normal cell wall biosynthesis and cell elongation. *Plant Cell* **13**, 807-827.

Byrne, M.E., Barley, R., Curtis, M., Arroyo, J.M., Dunham, M., Hudson, A., and Martienssen, R.A. (2000). *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis. Nature* **408**, 967-971.

Byrne, M.E., Timmermans, M. T., Kidner, C., and Martienssen, R. (2001). Development of leaf shape. *Curr. Opin. Plant Biol.* **4**, 38-43.

Candela, H., Martínez-Laborda, A., and Micol, J.L. (1999). Venation pattern formation in *Arabidopsis thaliana* vegetative leaves. *Dev. Biol.* **205**, 205-216.

Chaudhury, A.M., Letham, S., Craig, S, and Dennis, E.S. (1993). *amp1* – a mutant with high cytokinin levels and altered embryonic pattern, faster vegetative growth, constitutive photomorphogenesis and precocious flowering. *Plant J.* **4**, 907-916.

Chien, J.C., and Sussex, I.M. (1996). Differential regulation of trichome formation on the adaxial and abaxial leaf surfaces by gibberellins and photoperiod in *Arabidopsis thaliana* (L.) Heynh. *Plant Physiol.* **111**, 1321-1328.

Cho, H.-T., and Cosgrove, D. (2000). Altered expression of expansin modulates leaf growth and pedicel abscission in *Arabidopsis thaliana. Proc. Natl. Acad. Sci. U.S.A.* **97**, 9783-9788.

Choe, S., Tanaka, A., Noguchi, T., Fujioka, S., Takatsuto, S., Ross, A.S., Tax, F.E., Yoshida, S., and Feldmann, K.A. (2000). Lesions in the sterol $^{3}7$ reductase gene of *Arabidopsis* cause dwarfism due to a block in brassinosteroid biosynthesis. *Plant J.* **21**, 431-443.

Chuck, G., Lincoln, C., and Hake, S. (1996). *KNAT1* induces lobed leaves with ectopic meristems when overexpressed in Arabidopsis. *Plant Cell* **8**, 1277-1289.

Cockcroft, C.E., den Boer, B.G.W., Healy, J.M.S., and Murray, J.A.H. (2000). Cyclin D control of growth rate in plants. *Nature* **405**, 575-579.

Cyr, R.J. (1994). Microtubules in plant morphogenesis: role of the cortical array. *Annu. Rev. Cell Biol.* **10**, 153-180.

Dale, J.E. (1988). The control of leaf expansion. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **39**, 267-295

Dengler, N., and Kang, J. (2001). Vascular patterning and leaf shape. *Curr. Opin.Plant Biol.* **4**, 50-56.

Dengler, N., and Tsukaya, H. (2001). Leaf morphogenesis in dicotyledons: current issues. *Int. J. Plant Sci.* **162**, 459-464.

Donnelly, P.M., Bonetta, D., Tsukaya, H., Dengler, R., and Dengler, N.G. (1999). Cell cycling and cell enlargement in developing leaves of *Arabidopsis. Dev. Biol.* **215**, 407-419.

Eshed, Y., Baum, S. F., and Bowman, J. L. (1999). Distinct mechanisms promote polarity establishment in carpels of *Arabidopsis. Cell* **99**, 199-209.

Feldmann, K.A., Marks, M.D., Christianson, M.L., and Quatrano, R.S. (1989). A dwarf mutant of *Arabidopsis* generated by T-DNA insertion mutagenesis. *Science* **243**, 1351-1354.

Felix, G., Altmann, T., Uwer, U., Jessop, A., Willmitzer, L., and Morris, P.-C. (1996). Characterization of *waldmeister,* a novel developmental mutant in *Arabidopsis thaliana. J. Exp. Bot.* **47**, 1007-1017.

Fletcher, J.C., Brand, U., Running, M.P., Simon, R. and Meyerowitz, E.M. (1999). Signaling of cell fate decisions by *CLAVATA3* in *Arabidopsis* shoot meristems. *Science* **283:** 1911-1914.

Fujioka, S., Li, J., Choi, Y.H., Seto, H., Takatsuto, S., Noguchi, T., Watanabe, T., Kuriyama, H., Yokota, T., Chory, J., and Sakurai, A. (1997). The Arabidopsis *deetiolated2* mutant is blocked early in brassinosteroid biosynthesis. *Plant Cell* **9**, 1951-1962.

Furutani, I., Watanabe, Y., Prieto, R., Masukawa, M., Suzuki, K., Naoi, K., Thitamadee, S., Shikanai, T., and Hashimoto, T. (2000). The *SPIRAL* genes are required for directional control of cell elongation in *Arabidopsis thaliana. Development* **127**, 4443-4453.

Goliber, T., Kessler, S., Chen, J.-J., Bharathan, G., and Sinha, N. (1999). Genetic, molecular, and morphological analysis of compound leaf development. *Curr. Topics Dev. Biol.* 43, 259-290.

Goodrich, J., Puangsomlee, P., Martin, M., Long, D., Meyerowitz, E.M., Coupland, G. (1997). A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. *Nature*. **386**, 44-51.

Granger, C.L., Callos, J.D., and Medford, J.I. (1996). Isolation of an *Arabidopsis* homologue of the maize homeobox *Knotted-1* gene. *Plant Mol. Biol.* **31**, 373-378.

Ha, C.-M., Kim, G.-T., Kim, B.C., Jun, J.H., Lee, U., Ueno, Y., Machida, Y., Tsukaya, H., and Nam, H. (2001). The *BLP* gene is required for maintenance of the determinate state of leaves. *Abstract of the 46th Int. NIBB Conf.:* "Genetics and Epigenesis". (Okazaki, Japan) no. 62.

Hanson, J., Johannesson, H., and Engstrom, P. (2001). Sugar-dependent alterations in cotyledon and leaf development in transgenic plants expressing the HDZip gene *ATHB13*. *Plant Mol. Biol.* **45**, 247-262.

Hara, N. (1957). On the types of the marginal growth in dicotyledonous foliage leaves. *Bot. Mag. Tokyo.* **70,** 108-114.

Hara, N. (1959). Marginal growth of leaves. *Nature* **183,** 1409-1410.

Hedden, P. (1999). Recent advances in gibberellin biosynthesis. J. Exp. Bot. **50,** 553-563.

Hu, Y., Bao, F., and Li, J. (2000). Promotive effect of brassinosteoids on cell division involves a distinct *CycD3*-induction pathway in *Arabidopsis.* Plant J. **24,** 693-701.

Ito, T., Kim, G.-T., and Shinozaki, K. (2000). Disruption of an *Arabidopsis* cytoplasmic ribosomal protein S13-homologous gene by transposon-mediated mutagenesis causes aberrant growth and development. Plant J. **22,** 257-264.

Jackson, D., Veit, B., and Hake, S. (1994). Expression of maize *KNOTTED1*-related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. Development **120,** 405-413.

Jenks, M.A., Rashotte, A.M., Tuttle, H.A., and Feldmann, K.A. (1996). Mutants in *Arabidopsis thaliana* altered in epicuticular wax and leaf morphology. Plant Physiol. **110,** 377-385.

Kaplan, D.R., and Hagemann, W. (1991). The relationship of cell and organism in vascular plants. BioScience **41,** 693- 703.

Kauschmann, A., Jessop, A., Koncz, C., Szekeres, M., Willmitzer, L., and Altmann, T. (1996). Genetic evidence for an essential role of brassinosteroids in plant development. Plant J. **9,** 701-713.

Kerstetter, R.A., Bollman, K., Taylor, A., Bomblies, K., and Poethig, S. (2001). *KANADI* regulates organ polarity in *Arabidopsis.* Nature **411,** 706-709.

Kerstetter, R., Vollbrecht, E., Lowe, B., Veit, B., Yamaguchi, J., and Hake, S. (1994). Sequence analysis and expression patterns divide the maize *knotted1*-like homeobox genes into two classes. Plant Cell **6,** 1877-1887.

Kim, G.-T., and Tsukaya, H. (2001). Cytochrome P450s and their roles in the regulation of the biosynthesis of plant hormones. Plant Cell Physiol. (in press).

Kim, G.-T., Tsukaya, H., and Uchimiya, H. (1998a). The *ROTUNDIFOLIA3* gene of *Arabidopsis thaliana* encodes a new member of the cytochrome P-450 family that is required for the regulated polar elongation of leaf cells. Genes Dev. **12,** 2181-2191.

Kim, G.-T., Tsukaya, H., and Uchimiya, H. (1998b). The *CURLY LEAF* gene controls both division and elongation of cells during the expansion of the leaf blade in *Arabidopsis thaliana*. Planta **206,** 175-183.

Kim, G.-T., Tsukaya, H., Saito, Y., and Uchimiya, H. (1999). Changes in the shapes of leaves and flowers upon overexpression of cytochrome P450 in *Arabidopsis.* Proc. Natl. Acad. Sci. U.S.A. **96,** 9433-9437.

Koornneef, M., van Eden, J., Hanhart, C.J., Stam, P., Braaksma, F.J. , and Feenstra, W.J. (1983). Linkage map of *Arabidopsis thaliana*. J. Hered. **74,** 265-272.

Lake, J.A., Quick, W.P., Beerling, D.J., and Woodward, F.I. (2001). Signals from mature to new leaves. Nature **411,** 154.

Laux, T., Mayer, K.F.X., Berger, J., and Jürgens, G. (1996). The *WUSCHELL* gene is required for shoot and floral meristem integrity in *Arabidopsis.* Development **122,** 87-96.

Lee-Chen, S., and Steinitz-Sears, L.M. (1967). The location of linkage groups in *Arabidopsis thaliana.* Can. J. Genet. Cytol. **9,** 381-384.

Leyser, H.M.O., Lincoln, C.A., Timpte, C., Lammer, D., Turner, J., and Estelle, M. (1993). *Arabidopsis* auxin-resistance gene *AXR1* encodes a protein related to ubiquitin-activating enzyme E1. Nature **364,** 161-164.

Li, J., and Chory, J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell **90,** 929-38.

Lightner, J., James, D.W. Jr., Dooner, H.K., and Browse, J. (1994). Altered body morphology is caused by increased levels in a mutant of *Arabidopsis.* Plant J. **6,** 401-412.

Lincoln, C., Britton, J.H., and Estelle, M. (1990). Growth and development of the *axr1* mutants of *Arabidopsis.* Plant Cell **2,** 1071-1080.

Lincoln, C., Long, J., Yamaguchi, J., Serikawa, K., and Hake, S. (1994). A *Knotted1*-like homeobox gene in *Arabidopsis* is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants. Plant Cell **6,** 1859-1876.

Long, J.A., and Barton, M.K. (1998). The development of apical embryonic pattern in *Arabidopsis.* Development **125,** 3027-3035.

Long, J.A., Moan, E.I., Medford, J.I. and Barton, M.K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the *STM* genes of *Arabidopsis.* Nature **379,** 66-69.

Lotan, T., Ohto, M., Matsudaira Yee, K., West, M.A.L., Lo, R., Kwong, R.W., Yamagishi, K., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (1998). *Arabidopsis LEAFY COTYLEDON1* is sufficient to induce embryo development in vegetative cells. Cell **93,** 1195-1205.

Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., and Barton, M.K. (1999). The *PINHEAD/ZWILLE* gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the *ARGONAUTE1* gene. Developement **126,** 469-481.

Maksymowych, R. (1963). Cell division and cell elongation in leaf development of *Xanthium pennsylvanicum*. Amer. J. Bot. **50,** 891-901.

Marx, G.A. (1983). Developmental mutants in some annual seed plants. Annu. Rev. Plant Physiol. **34,** 389-417.

Meyerowitz, E.M. (1997). Genetic control of cell division patterns in developing plants. Cell **88,** 299-308.

McConnell, J.R., and Barton, M.K. (1998). Leaf polarity and meristem formation in *Arabidopsis.* Development **125,** 2935-2942.

McConnell, J.R., Emery, J., Eshed, Y., Bao, N., Bowman, J., and Barton, M.K. (2001). Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots. Nature **411,** 709-713.

Meisel, L., Xie, S. and Lam, E. (1996). *lem7,* a novel temperature-sensitive *Arabidopsis* mutation that reversivbly inhibits vegetative development. Dev. Biol. **179,** 116-134.

Meinke, D.W. (1992). A homoeotic mutant of *Arabidopsis thaliana* with leafy cotyledons. Science **258,** 1647-1650.

Meyerowitz, E.M., and Pruitt, R.E. (1985). *Arabidopsis thaliana* and plant molecular genetics. Science **229,** 1214-1218.

Mizukami, Y., and Fischer, R.L. (2000). Plant organ size control: *AINTEGUMENTA* regulates growth and cell numbers during organogenesis. Proc. Natl. Acad. Sci. U.S.A. **97,** 942-947.

Nagatani, A., Chory, J., and Furuya, M. (1991). Phytochrome B is not detectable in the *hy3* mutant of *Arabidopsis*, which is deficient in responding to end-of-day far-red light treatments. Plant Cell Physiol. **32,** 1119-1122.

Nagatani, A., Reed, J.W., and Chory, J. (1993). Isolation and initial characterization of *Arabidopsis* mutants that are deficient in phytochrome A. Plant Physiol. **102,** 269-277.

Nagpal, P., Walker, L.M., Young, J.C., Sonawala, A., Timpte, C., Estelle, M., and Reed, J.W. (2000). *AXR2* encodes a member of the Aux/IAA protein family. Plant Physiol. **123,** 563-573.

Nicol, F., His, I., Jauneau, A., Vernhettes, S., Canut, H., and Hofte, H. (1998). A plasma membrane-bound putative endo-1,4-b-D-glucanase is required for normal wall assembly and cell elongation in *Arabidopsis*. EMBO J. **17,** 5563 – 5576.

Noguchi, T., Fujioka, S., Takatsuto, S., Sakurai, A., Yoshida, S., Li, J., and Chory, J. (1999). *Arabidopsis det2* is defective in the conversion of (24R)-24-methylcholest-4-En-3-one to(24R)-24-methyl-5alpha-cholestan-3-one in brassinosteroid biosynthesis. Plant Physiol. **120,** 833-840.

Ori, N., Eshed, Y., Chuck, G., Bowman, J.L., and Hake, S. (2000). Mechanisms that control *knox* gene expression in the *Arabidopsis* shoot. Development **127,** 5523-5532.

Poethig, R.S. (1997). Leaf morphognesis in flowering plants. *Plant Cell* **9**, 1077-1087.

Poethig, R.S., and Sussex, I.M. (1985). The developmental morphology and growth dynamics of the tobacco leaf. Planta **165,** 158-169.

Prigge, M.J. and Wagner, D.R. (2001). The Arabidopsis *SERRATE* gene encodes a zinc-finger protein required for normal shoot development. Plant Cell **13,** 1263-1279.

Pyke, K.A., Marrison, J.L., and Leech, R.M. (1991). Temporal and spatial development of the cells of the expanding first leaf of *Arabidopsis thaliana* (L.) Heynh. J. Exp. Bot. **42,** 1407-1416.

Ratcliffe, O.J., Riechmann, J.L., and Zhang, J.Z. (2000). *INTERFASCICULAR FIBERLESS1* is the same gene as *REVOLUTA.* Plant Cell **12,** 315-317.

Rédei, G.P. (1962). Single locus heterosis. *Z. Vererbungs.* **93,** 164-170.

Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M., and Chory, J. (1993). Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. Plant Cell **5,** 147-157.

Röbbelen, G. (1957). Über heterophyllie bei *Arabidopsis thaliana* (L.) Heynh. Ber. Dt. Bot. Ges. **70,** 39-44.

Roffer-Turner, M., and Napp-Zinn, K. (1979). Investigations on leaf structure in several genotypes of *Arabidopsis thaliana* (L.) Heynh. Arabidopsis Inf. Ser. **16,** 94-98.

Rupp, H.-M., Frank, M., Werner, T., Strnad, M., and Schmüling, T. (1999). Increased steady state mRNA levels of the *STM* and *KNAT1* homeobox genes in cytokinin overproducing *Arabidopsis thaliana* indicate a role for cytokinins in the shoot apical meristem. Plant J. **18,** 557-563.

Sakamoto,T., Kamiya, N., Ueguchi-Tanaka, M., Iwahori, S., and Matsuoka, M. (2001). KNOX homeodomain protein directly suppresses the expression of a gibberellin biosynthetic gene in the tobacco shoot apical meristem. Genes Dev. **15,** 581-590.

Sawa, S., Watanabe, K., Goto, K., Kanaya, E., Morita, E.H., and Okada, K. (1999). *FILAMENTOUS FLOWER,* a meristem and organ identity gene of *Arabidopsis,* encodes a protein with a zinc finger and HMG-related domains. Genes Dev. **13,** 1079-1088.

Semiarti, E., Ueno, Y., Tsukaya, H., Iwakawa, H., Machida, C., and Machida, Y. (2001). The *ASYMMETRIC LEAVES2* gene of *Arabidopsis thaliana* regulates formation of a symmetric lamina, establishment of venation and repression of meristem-related homeobox genes in leaves. Development **128,** 1771-1783.

Serikawa, K. A., Martinez-Laborda, A., and Zambryski, P. (1996). Three *knotted1*-like homeobox genes in *Arabidopsis.* Plant Mol. Biol. **32,** 673-683.

Serikawa, K. A., Martinez-Laborda, A., Kim, H.-S., and Zambryski, P. (1997). Localization of expression of *KNAT3*, a class 2 *knotted1*-like gene. Plant J. **11,** 853-861.

Serikawa, K.A. and Zambryski, P.C. (1997). Domain exchanges between KNAT3 and KNAT1 suggest specificity of the kn1-like homeodomains requires sequences outside of the third helix and N-terminal arm of the homeodomain. Plant J. **11,** 863-869.

Serrano-Cartagena, J., Candela, H., Robles, P., Ponce, M.R., Pérez-Pérez, J.M., Piqueras, P., and Micol, J.L. (2000). Genetic analysis of *incurvata* mutants reveals three independent genetic operations at work in Arabidopsis leaf morphogenesis. Genetics **156,** 1363-1377.

Serrano-Cartagena, J., Robles, P., Ponce, M.R., and Micol, J.L. (1999). Genetic analysis of leaf form mutants from the *Arabidopsis* Information Service collection. *Mol. Gen. Genet.* **261,** 725-739.

Shevell., D.E., Leu, W.-M., Gillmor, C.S., Xia, G., Feldmann, K.A., and Chua, N.-H. (1994). *EMB30* is essential for normal cell division, cell expansion, and cell adhesion in Arabidopsis and encodes a protein that has similarity to Sec7. Cell **77,** 1051-1062.

Shibaoka, H. (1994). Plant hormone-induced changes in the orientation of cortical microtubules: alterations in the cross-linking between microtubules and the plasma membrane. Annu. Rev. Plant Physiol. Plant Mol. Biol. **45,** 527-544.

Siegfried, K.R., Eshed, Y., Baum, S.F., Otsuga, D., Drews, G.N., and Bowman, J.L. (1999). Members of the *YABBY* gene family specify abaxial cell fate in *Arabidopsis.* Development **126,** 4117-4128

Sinha, N. (1997) Simple and compound leaves: reduction or multiplication? Trends Plant Sci. **2,** 396-402.

Sinha, N. (1999) Leaf development in angiosperms. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 4117-4128.

Smith, L.G., Greene, B., Veit, B., and Hake, S. (1992). A dominant mutation in the maize homeobox gene, *Knotted-1*, causes its ectopic expression in leaf cells with altered fates. Development **116,** 21-30.

Smith, L.G., and Hake, S. (1992). The initiation and determination of leaves. Plant Cell **4,** 1017-1027.

Smith, L.G., and Hake, S. (1993). Molecular genetic approaches to leaf development: *Knotted* and beyond. Can. J. Bot. **72,** 617-625.

Sultan, S.E. (2000). Phenotypic plasticity for plant development, function and life history. Trends Plant Sci. **5,** 537-542.

Szekeres, M., Németh, K., Koncz-Kálmán, Z., Mathur, J., Kauschmann, A., Altmann, T., Rédei, G.P., Nagy, F., Schell, J., and Koncz, C. (1996). Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis.* Cell **85,** 171-182.

Steeves, T.A., and Sussex, I.M. (1989). "Patterns in Plant Development", 2nd ed., Cambridge University Press, Cambridge.

Sun, T.-P. (2000). Gibberellin signal transduction. Curr. Opin. Plant Biol. **3,** 374-380.

Takahashi, T., Gasch, A., Nishizawa, N., and Chua, N.-H. (1995). The *DIMINUTO* gene of *Arabidopsis* is involved in regulating cell elongation. Genes Dev. **9,** 97-107

Talbert, P.B., Alder, H.T., Parks, D.W., and Comail, L. (1995). The *REVOLUTA* gene is necessary for apical meristem development and for limiting cell divisions in the leaves and stems of *Arabidopsis thaliana.* Development **121,** 2723-2735.

Telfer, A., Bollman, K.M., and Poethig, R.S. (1997). Phase change and the regulation of trichome distribution in *Arabidopsis thaliana*. Development **124,** 645-654.

Timpte, C.C., Wilson, A.K., and Estelle, M. (1992). Effects of the *axr2* mutation of *Arabidopsis* on cell shape in hypocotyl and inflorescence. Planta **188,** 271-278.

Timpte, C., Lincoln, C., Pickett, F.B., Turner, J., and Estelle, M. (1995). The *AXR1* and *AUX1* genes of *Arabidopsis* function in separate auxin-response pathways. Plant J. **8,** 561-569.

Tsiantis, M., Schneeberger, R., Golz, J.F., Freeling, M., and Langdale, J.A. (1999). The maize *rough sheath2* gene and leaf development programs in monocot and dicot plants. Science **284,** 154-156.

Tsuge, T., Tsukaya, H., and Uchimiya, H. (1996). Two independent and polarized processes of cell elongation regulate leaf blade expansion in *Arabidopsis thaliana* (L.) Heynh. Development **122,** 1589-1600.

Tsukaya, H. (1995a). Developmental genetics of leaf morphogenesis in dicotyledonous plants. J. Plant Res. **108,** 407-416.

Tsukaya, H. (1995b). The genetic control of morphogenesis in *Arabidopsis* and its relevance to the development of biodiversity. In Biodiversity and Evolution, R. Arai, M. Kato, and Y. Doi, eds (Tokyo, The National Science Museum Foundation). pp. 253-265.

Tsukaya, H. (1998). Genetic evidence for polarities that regulate leaf morphogenesis. J. Plant Res. **111,** 113-119.

Tsukaya, H. (2000). The role of meristematic activities in the formation of leaf blades. J. Plant Res. **113,** 119-126.

Tsukaya, H. (2001). Interpretation of mutants in leaf morphology: genetic evidence for a compensatory system in leaf morphogenesis that provides a new link between Cell and Organismal theory. Int. Rev. Cytol. (**in press**)

Tsukaya, H., Inaba-Higano, K., and Komeda, Y. (1995). Phenotypic characterization and molecular mapping of *acaulis2* mutant with flower stalks of much reduced length in *Arabidopsis thaliana*. Plant Cell Physiol. **36,** 239-246.

Tsukaya, H., Naito, S., Rédei, G.P., and Komeda, Y. (1993). A new class of mutations in *Arabidopsis thaliana, acaulis1,* affecting the development of both inflorescences and leaves. Development **118,** 751-764.

Tsukaya, H., Shoda, K., Kim, G.-T., and Uchimiya, H. (2000). Heteroblasty in *Arabidopsis thaliana* (L.) Heynh. Planta **210,** 536-542.

Tsukaya, H., Tsuge, T., and Uchimiya, H. (1994). The cotyledon: a superior system for studies of leaf development. Planta **195,** 309-312.

Tsukaya, H., and Uchimiya, H. (1997)**.** Genetic analyses of developmental control of serrated margin of leaf blades in Arabidopsis — Combination of mutational analysis of leaf morphogenesis with characterization of a specific marker gene, which expresses in hydathodes and stipules in *Arabidopsis*. Mol. Gen. Genet. **256,** 231-238.

Ullah, H., Chen, J.-G., Young, J.C., Im, K.-H., Susman, M.R., and Jones, A.M. (2001). Modulation of cell proloferation by heterotrimeric G protein in *Arabidopsis.* Science **292,** 2066-2069.

Van Lijsebettens, M., and Clarke, J. (1998). Leaf development in *Arabidopsis.* Plant Physiol. Biochem. **36,** 47-60.

Van Lijsebettens, M., Vanderhaeghen, R., and Van Montague, M. (1991). Insertional mutagenesis in *Arabidopsis thaliana*: isolation of a T-DNA-linked mutation that alters leaf morphology. Theor. Appl. Genet. **81,** 277-284.

Van Lijsebettens, M., Vanderhaeghen, R., De Block, M., Bauw, G., Villarroel, R., and Van Montagu, M. (1994). An S18 ribosomal protein gene copy at the *Arabidopsis PFL* locus affects plant development by its specific expression in meristems. EMBO J. **13,** 3378-3388.

Van Volkenburgh, E. (1999). Leaf expansion – an integrating plant behavior. Plant Cell Environ. **22,** 1463-1473.

Van der Graaff, E., Dulk-Ras, A.D., Hooykaas, P.J.J., and Keller, B. (2000). Activation tagging of the *LEAFY PETIOLE* gene affects leaf petiole development in *Arabidopsis thaliana.* Development **127,** 4971-4980.

Vollbrecht, E., Veit, B., Sinha, N., and Hake, S. (1991). The developmental gene *Knotted-1* is a member of a maize homeobox gene family. Nature **350,** 241-243.

Waites, R., and Hudson, A. (1995). *phantastica*: a gene required for dorsoventrality of leaves in *Antirrhinum majus*. Development **121,** 2143-2154.

Waites, R., Selvadurai, H.R.N., Oliver, I.R., and Hudson, A. (1998). The *PHANTASTICA* gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum.* Cell **93,** 779-789.

Wang, H., Zhou, Y., Gilmer, S., Whitwill, S., and Fowke, L.C. (2000). Expression of the plant cyclin-dependent kinase inhibitor ICK1 affects cell division, plant growth and morphology. Plant J. **24,** 613-623.

Wang, X-Q., Ullah, H., Jones, A.M., and Assmann, S.M. (2001). G protein regulation of ion channels and abscisic acid signaling in *Arabidopsis* guard cells. Science **292,** 2070-2072.

West, M.A.L., Yee, K.M., Danao, J., Zimmerman, J.L., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (1994). *LEAFY COTYLEDON1*is an essential regulator of late embryogenesis and cotyledon identity in Arabidopsis. Plant Cell **6,** 1731-1745.

Williams, R.W. (1998). Plant homeobox genes: many functions stem from a common motif. Bioessays **20,** 280-282.

Yadegari, R., de Paiva, G.R., Laux, T., Koltunow, A.M., Apuya, N., Zimmerman, J.L., Fischer, R.L., Harada, J.J., and Goldberg, R.B. (1994). Cell differentiation and morphogenesis are uncoupled in Arabidopsis *raspberry* embryos. Plant Cell **6,** 1713-1729.

Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A., and Meyerowitz, E.M. (1990). The protein encoded by the *Arabidopis* homeotic gene *agamous* resembles transcriptional factors. Nature **346,** 35-39.

Zhong, R., Taylor, J.J., and Ye, Z.-H. (1997). Disruption of interfascicular fiber differentiation in an Arabidopsis mutant. Plant Cell **9,** 2159-2170.

Zhong, R., and Ye, Z.-H. (1999). *IFL1,* a gene regulating interfascicular fiber differentiation in Arabidopsis, encodes a homeodomain-leucine zipper protein. Plant Cell **11,** 2139-2152.