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Hirokazu Tsukaya

National Institute for Basic Biology/Center for Integrated Bioscience, Okazaki National Institutes, Myodaiji-cho, Okazaki 444-8585, Japan; Additional affiliations: ‘Form and Function’, PRESTO, Japan Science and Technology Corporation, Japan; School of Advanced Sciences, The Graduate University for Advanced Studies, Shonan Villege, Hayama, Kanagawa 240-0193, Japan; fax: +81-564-55-7512; e-mail: tsukaya@nibb.ac.jp

Abstract

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,
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 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 (pdf) 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 thaliana) 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 elongation 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 KNOTTED genes around leaf primordia in Arabidopsis (e.g., Byrne et al., 2000).

Since leaf initiation is the most striking event that occurs 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 dorsiAVentrality, 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 Schiefelbein, 1997; Fletcher et al., 1998; Moussian et al., 1998; Lenhard and Laux, 1999; Trottochaud 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 (warn), Felix et al. (1996) observed a number of defects in shoots, namely, altered leaf shape, in addition to occurrence of aerial rosettes, fascination 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 KNOX 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
rough sheath2 \((rs2)\) gene of maize, a homolog of \textit{PHAN}, prevents the accumulation of \textit{kn1} mRNA in leaf primordia (Tsiantis et al., 1999). In \textit{Arabidopsis}, the \textit{ASYMMETRIC LEAVES (AS)} gene is a homolog of the \textit{PHAN} and \textit{rs2} genes and the product of the \textit{AS1} gene is expressed in the subepidermal cells of leaves and in the SAM (Byrne et al., 2000). A loss-of-function type of \textit{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 \textit{AS1} gene acts negatively to regulate the class 1 \textit{KNOX} genes of \textit{Arabidopsis}, namely, \textit{KNAT1} and \textit{KNAT2} (Byrne et al., 2000; Semiarti et al., 2001). Expression of the \textit{AS1} gene is, moreover, negatively regulated by the \textit{SHOOT MERISTEMLESS} gene in the SAM (Ori et al., 2000; Byrne et al., 2000).

The pattern of expression of class I \textit{KNOX} genes in the SAM is regulated not only by the \textit{AS1} gene. Leaves of \textit{as2} mutant plants resemble those of the \textit{as1} mutant in shape (Tsukaya and Uchimiya, 1997; Ori et al., 2000; Semiarti et al., 2001; Fig. 5), and \textit{as2} plants also express class I \textit{KNOX} genes ectopically in their leaves (Ori et al., 2000; Semiarti et al., 2001). Analysis by reverse-transcriptase-PCR (RT-PCR) showed that \textit{as2} plants express \textit{KNAT1}, \textit{KNAT2} and \textit{KNAT6} genes in their young leaves, whereas the wild type does not express these \textit{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 \textit{KNOX} genes. They found, for example, that the \textit{serrata (se)} mutation enhances phenotypes of \textit{as1} and \textit{as2} plants. Moreover, the phenotypes of \textit{se as1/ as2} double mutants resemble that of transgenic \textit{Arabidopsis} that overexpresses the \textit{KNAT1} gene, with ectopic meristems on the sinuses of leaf blades (Ori et al., 2000). The \textit{SE} gene encodes a zinc-finger protein whose mRNA is expressed...
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 (pk1) 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 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.
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).
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(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 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).
REVOLUTA/INTERFASCICULAR FIBERLESS gene, which plays an important role in the meristematic activity in the axillary SAM and leaf primordia (Taiz 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 be 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),
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 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 circumference 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.
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 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.
phloem), and the polar arrangement of vascular tissue is regulated by the REVOLUTA/INTERFASCICULAR FIBERLESS gene, as mentioned above. It is noteworthy, 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).

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**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.
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-length 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 sterol 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.

The polarized expansion of plant cells is controlled by the orientation of cortical microtubules (MTs; for reviews, see Cyr, 1994; Shibatoka, 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 translated 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 petals and larger leaf blades than the wild type (Cho and Cosgrove, 2000).

Tsukaya et al. (1993) reported that acaulis (ac) 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; Fujisaka 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 (Leysen 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).
data). Recently, Lake et al. (2001) reported that signals perceived by old mature leaves, for example, light intensity and the concentration of CO₂, 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 polycorn 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.
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 in terms of the results of mutational analysis.

Identity of cotyledon in terms of heteroblasty

Heteroblasty or heterophyllly, 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 be 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

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**Figure 13.** Heteroblasty in Arabidopsis (Columbia wild type) under continuous light, 22°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.
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 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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