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Genetic and Epigenetic Control of Leaf Size and Shape

Kristiina Himanen • Getnet Dino Adem • Mieke Van Lijsebettens*

Department of Plant Systems Biology, Flanders Institute for Biotechnology (VIB) and Department of Molecular Genetics, Ghent University, Technologiepark, 927, B-9052 Gent, Belgium

Corresponding author: * **mieke.vanlijsebettens@psb.ugent.be**

ABSTRACT

Leaf shapes and sizes vary naturally from simple with smooth, serrated or lobed margins to compound with a few or a lot of leaflets. Simple leaves develop through gradients of cell division and cell expansion from tip to base, resulting in a fully differentiated mature leaf without meristematic activity, referred to as a determinate structure. Cell numbers and cell expansion influence leaf size and shape as observed by manipulation of the core cell cycle or cell wall extensibility. However, mechanisms exist that compensate changes in leaf growth by affecting cell expansion or number, which indicates that leaf size is also under supracellular control. Foliar morphology is used for classification in botany, demonstrating its genetic and evolutionary basis. A developmental biology approach is taken to identify the molecular control of leaf size and shape by using a limited number of model species. Mutational and transgene analysis in *Arabidopsis thaliana* has uncovered more than 100 loci important for simple leaf development. Regulatory genes, such as transcription factors, have been shown to regulate leaf growth and development, of which some might act upstream of the hormonal responses and core cell cycle. Chromatin modification complexes are involved in the control of leaf growth and might form the interface with developmental and environmental cues to influence leaf formation – a phenomenon known as leaf plasticity. In contrast to simple leaves, compound leaves develop discernable meristems that will form leaflets along the rachis. Molecular-genetic work in snapdragon (*Antirrhinum majus*), tomato (*Solanum lycopersicum*), and pea (*Pisum sativum*) identified independent molecular pathways for compound leaf development. These regulatory pathways have previously been shown to be important for meristem identity and suggest that compound leaves can be considered as transitional forms from determinate simple leaves to indeterminate shoots. The use of developmental genes for applications in agriculture, horticulture and ornamentals will be discussed.

Keywords: simple and compound leaves, leaf growth, cell cycle, forward genetics, chromatin modifying complexes
Abbreviations: **ABA**, abscisic acid; **CAK**, CDK-activating kinase; **CDK**, cyclin-dependent kinase; **CKI**, CDK inhibitor; **CKS**, CDK subunit; **EMS**, ethyl methanesulfonate; **GA**, gibberellic acid; **GUS**, β-glucuronidase; **RAM**, root apical meristem; **SAM**, shoot apical meristem; **TALE-HD**, three-amino-acid loop extension-homeodomain protein

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THE ORIGIN OF LEAVES

The shoot apex

During embryogenesis, a rudimentary plant axis is established with a shoot apical meristem (SAM) and a root apical meristem (RAM) (Weigel and Jürgens 2002). After embryogenesis, most plant organs are formed by the activities of these apical meristems. The meristems mediate indeterminate growth and local formative divisions establish cell lineage patterns of new organs. The SAM is a domain where stem cells are continuously produced and provide the material for the formation of new organs, such as leaves (**Fig. 1A**) (Bäurle and Laux 2003; Cole *et al.* 2006). After germination, the SAM starts to produce lateral organs and stem tissues that are organized in the so-called phytomers. The SAM consists of distinctive zones with respect to cell division activity and developmental destination, and has

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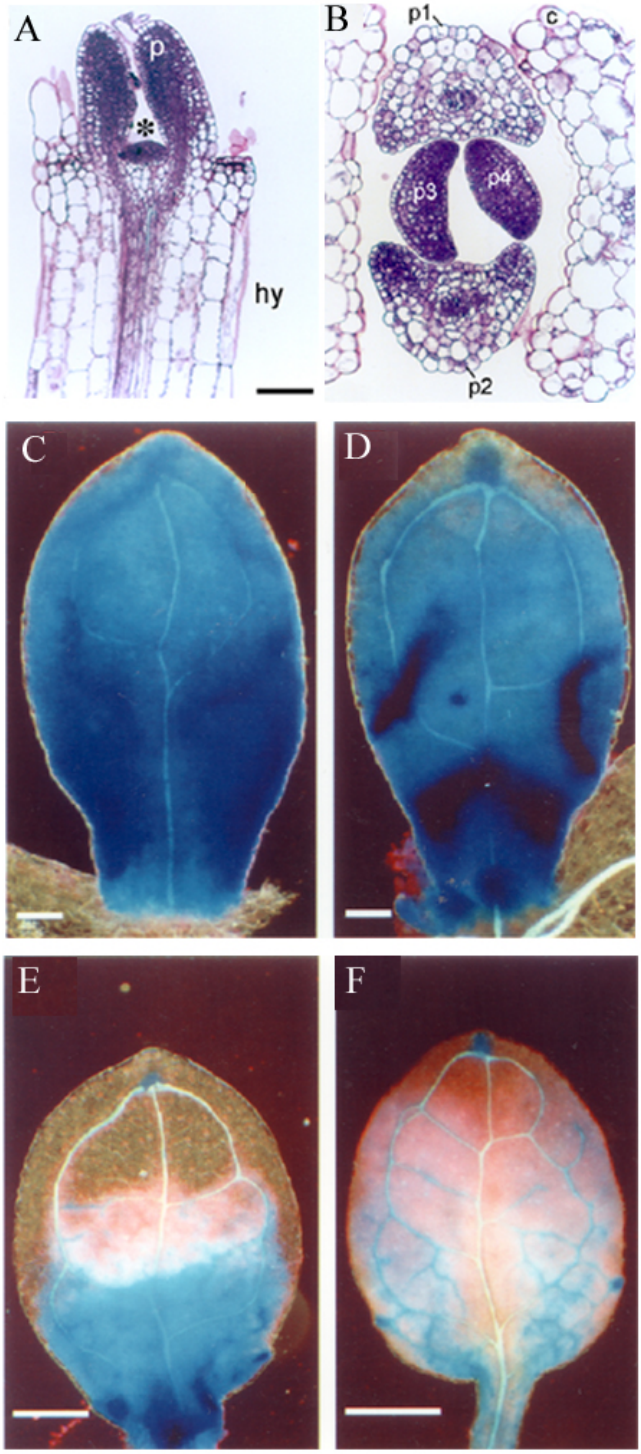

Fig. 1 Leaf initiation and proliferation. (**A**) Longitudinal section through a 6-day-old SAM of a wild-type plant. (**B**) Transverse section through a 12-day-old wild-type shoot apex. (**C-F**) Cell division profiles during the development of the first leaf in *Arabidopsis* ecotype C24 with a *pCYCB1;1-GUS* reporter line for measuring cell division activity (Van Lijsebettens and Clarke 1998; ©Elsevier, reprinted with kind permission). (**C**) 9-day-old, (**D**) 11-day-old, (**E**) 12-day-old, (**F**) 15-day-old first leaf. Bar = 50 μm [**A** and **B**], 100 μm [**C** and **D**], 500 μm [**E** and **F**].

been the subject of early developmental research and is still today (Sussex 1989; Laufs *et al*. 1998). In the central zone, stem cells stay in an indeterminate state and, upon division, they replenish themselves, but also produce daughter cells for the peripheral zone where they are recruited to initiate leaf primordia, or for the rib zone, where they contribute to the formation of internodium tissue. The stem cells are maintained in an undifferentiated state in specialized niches. Differentiation of the stem cell progeny outside the niche is affected by positional signals from more mature tissues. So far, genetic approaches have identified some key players in meristematic signaling; although most signals that relay the positional information await elucidation (Bäurle and Laux 2003). Clonal analysis has shown that stem cells are not permanent but can differentiate when they are displaced from the summit of the dome-shaped SAM, indicating that stem cell identity is not an inherent property of a given lineage but rather is conferred to cells by positional cues (Ruth *et al*. 1985). The SAM is also layered into L1, L2, and L3 that are the progenitor of epidermal tissues, of palisade and spongy parenchyma (and the sporogenic cells), and vascular tissues, respectively. Organ formation takes place in the peripheral zone of the SAM where a group of 15-30 cells derived from all three meristem layers become assigned to an incipient organ primordium (**Fig. 1A**) (Furner and Pumfrey 1992; Irish and Sussex 1992).

The origin of the SAM is embryonic, based on the expression of the *SHOOT MERISTEMLESS* (*STM*) gene starting from the late globular stage and marking the future SAM in the early heart-shaped embryo (Long and Barton 1998). *STM* is essential for SAM formation as shown by the *stm* knockout phenotype that produces seedlings without SAM (Barton and Poethig 1993; Long *et al*. 1996). *STM* encodes a KNOX domain/three-amino-acid loop extension (TALE)-HD-type transcription factor (Cole *et al*. 2006) and probably acts as transcriptional regulator in promoting SAM development and maintenance (Endrizzi *et al.* 1996). The TALE-HD factors have DNA binding activity because they were shown to interact with other groups of TALE-HD proteins in animals (Penkov *et al*. 2000). In plants, evidence is accumulating that KNOX/TALE-HD proteins interact with another class of TALE-HD gene products, the BEL1-like homeodomain (BLH) proteins (Bellaoui *et al.* 2001; Smith and Hake 2003; Bhatt *et al*. 2004). One of the first signs of organ initiation is the down-regulation of *STM* expression in the organ founder cells, while maintaining the expression in other parts of the meristem (Long *et al*. 1996), presumebly allowing the onset of *ASYMMETRIC LEAVES1 (AS1)* and *AS2* gene expression at leaf initiation sites of the SAM; in turn these genes repress meristematic cell fate by down-regulating the *KNOTTED*-like genes, *KNAT1*, *KNAT2*, and *KNAT6* in *Arabidopsis thaliana* that are also expressed in specific domains of the SAM (Lincoln *et al*. 1994; Byrne *et al.* 2000, 2002). How *STM* expression is down-regulated at the site of organ intiation remains to be unravelled (Bäurle and Laux 2003). The *STM* gene is the ortholog of the maize (*Zea mays*) *KNOTTED1* (*KN1*) gene that, upon ectopic expression in maize leaves, reverts the determinate to the indeterminate state, resulting in the production of knots (Vollbrecht *et al*. 1991). KN1 was the first plant protein whose plasmodesmal trafficking has been identified (Lucas 1995); that report emphasizes the importance of plasmodesmal cell-to-cell communication in developmental processes.

A genetic model has been proposed for self-regulation of the SAM. The *CLAVATA* (*CLV*) genes are responsible for the repression of growth in the central zone (Clark *et al.* 1997; Fletcher *et al.* 1999; Brand *et al.* 2000) and encode components of a signaling cascade that regulates *WUSCHEL* (*WUS*) activity (Laux *et al*. 1996; Trotochaud *et al*. 1999; Schoof *et al*. 2000). WUS is a homeodomain protein that keeps stem cells in their indeterminate state through a negative feedback loop with *CLV3* (Mayer *et al.* 1998). Instead of self-maintaining stem cells, cells in the apex differentiate in the *wus* mutant (Laux *et al*. 1996). The WUS expression domain, just beneath the stem cell zone is called the "organizing center" and is comparable to the quiescent center in the RAM (van den Berg *et al.* 1997; for review, see Weigel and Jürgens 2002; Rademacher and Weijers 2007).

Leaf initiation from the SAM

Leaf primordia originate at the SAM peripheral zone and have a multicellular origin because cells are recruited from

different SAM layers (**Fig. 1A, 1B**). Initiation of leaf requires the coordination of a group of founder cells in the SAM and cytoplasmic continuity through plasmodesmata has been postulated to play a role in the supracellular control of morphogenesis (Lucas 1995). A leaf primordium is initiated by periclinal cell division in the L2 layer, generating a new axis (proximal-distal) of growth away from the SAM (Medford *et al*. 1992). Upper and lower epidermis are formed by anticlinal division of the L1 layer and periclinal and anticlinal divisions of the L2 layer give rise to the inner tissues of the leaf (Furner and Pumfrey 1992). At emergence (4 days after sowing in *Arabidopsis*), leaf primordia have radial symmetry and immediately acquire dorsiventral asymmetry by flattening the side that faces the SAM, by formation of the first trichomes at the dorsal tip, and of small structures (stipules) at the base of the dorsal side that quickly degenerate (Van Lijsebettens and Clarke 1998).

Leaves have a precisely determined position relative to one another. This pattern or phyllotaxis has been laid down in the SAM (Irish and Sussex 1992; Medford *et al*. 1992). In *Arabidopsis*, the first leaves are opposed to each other, the third leaf is perpendicular to the axis formed by the first two, and the fourth leaf forms at an angle of 137° and is the start of the spiral phyllotaxis of the subsequent leaves (**Fig. 1B**). The sites of leaf initiation coincide with spots of auxin accumulation at the SAM and a model has been proposed for phyllotactic patterning by polar auxin transport (Reinhardt *et al*. 2003). The leaf initiation site is delineated by molecular markers, such as *AS1*, whose position at the periphery of the SAM depends on that of previously formed primordia. At the leaf inception site, no *STM* expression fits the exit from the proliferative to a differentiation state of the primordium founder cells in which *AS1* gene activity is derepressed. *AS1* in *Arabidopsis* (Byrne *et al*. 2000), its ortholog *ROUGH SHEATH2* (*RS2*) in maize (Timmermans *et al*. 1999; Tsiantis *et al*. 1999), and *PHANTASTICA* (*PHAN*) in snapdragon (Waites *et al*. 1998), the so-called *ARP* genes, are Myb-type transcription factors that are important for promoting adaxial fate in leaf primordia and whose function is conserved in monocots and dicots. Upon recessive mutation of the *ARP* genes, some of the *KNOX* genes are ectopically expressed in the leaves where they are normally inactive (Schneeberger *et al*. 1998; Byrne *et al*. 2000; Ori *et al*. 2000). A dominant gain-of-function mutation at the *KN1* locus changes the cell fate into indeterminate and creates indeterminate foci in the leaf lamina, indicating the importance of *KN1* gene down-regulation in the switch from the indeterminate to determinate state in lateral organ formation (Van Lijsebettens and Clarke 1998). Microsurgical experiments on the potato (*Solanum tuberosum*) shoot apex have shown that the SAM communicates with leaf primordia and that a signal is required to induce polarity in the leaf primordium. Incisions between the SAM and the primordium resulted in radial symmetrical rather than dorsiventral asymmetrical leaves (Sussex 1955). Although the signal is still unknown today, the genetic factors for polarity have been identified: these are *AS1* and the *HD-ZIPIII* transcription factors *PHABULOSA*, *PHAVOLUTA* and *REVOLUTA* (dorsal identity), and the *GARP* genes, *YABBY* and *FILAMENTOUS* (ventral identity) (Sawa *et al*. 1999; Siegfried *et al*. 1999; Kerstetter *et al*. 2001; McConnell *et al*. 2001). Knockout mutations in the polarity genes cause radialization of the leaf. The polarity genes are used as dorsal and ventral markers to study polarity in leaf mutants (Nelissen *et al*. 2003). Analysis of their genetic interactions resulted in a model for foliar dorsoventrality (for review, see Bowman 2004). In conclusion, a number of transcription factors determine the developmental dimensions of the simple leaf. Identification of the upstream signaling cascades that regulate and coordinate these transcription factors will be the focus of future research.

Compound or dissected leaves: A transition state to shoot

KNOX ectopic expression in several species

From a botanical point of view, the compound leaf has evolved earlier than the simple leaf and compound leaves have arisen several times in evolution. Therefore, the existence of different molecular mechanisms is to be expected that steer compound leaf formation. The ontogeny of compound leaves in dicotyledonous plants is distinct from that of simple leaves because the former have a marginal meristem, called blastozone, along both sides of the leaf primordium that produces leaflets (Hagemann and Gleissberg 1996). In contrast, simple leaves have no delineated meristem in their primordia, but instead develop by intercalary or diffuse growth as indicated by the gradient of the mitotic marker gene *pCYCB1;1-GUS* during leaf growth (**Fig. 1C-F**). The distinct meristems in the primordia of compound leaves reflect a phase of indeterminate growth and, therefore, compound leaves are considered as a transitional state

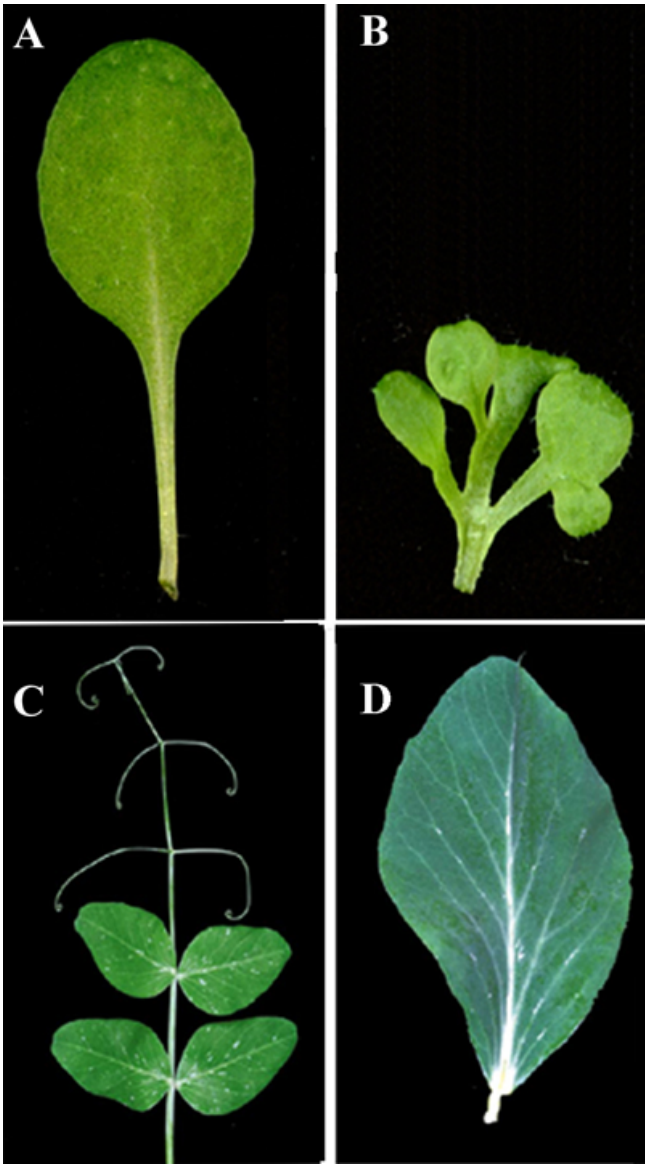

Fig. 2 Genes involved in compound leaf formation. (**A**) Wild-type *Arabidopsis* rosette leaf grown on Murashige and Skoog (MS) medium (**B**) Rosette leaf of *FIL>>BP Arabidopsis* plants expressing the *KNAT1/BP* gene under the *FIL* promoter, grown on MS medium (Hay *et al*. 2006; ©The Company of Biologists, reprinted with kind permission). (**C**) Wild-type pea leaf with two pairs of leaflets, three pairs of tendrils, and a terminal tendril. (**D**) *unifoliata* leaf in pea with a single leaflet (Hofer *et al*. 1997; ©Elsevier, reprinted with kind permission).

between the determinate state of the simple leaf and the indeterminate state of a shoot (Tsiantis and Hay 2003).

The indeterminate state of meristems is controlled by the KNOTTED1-type homeobox transcription factors, such as STM and KNAT in *Arabidopsis*. The function of *KNOX* genes is to maintain indeterminate cell state and to suppress differentiation. Repression of these genes at the leaf initiation sites of the SAM is essential in simple leaf formation in dicotyledonous and monocotyledonous plants and requires the activity of the *ARP* class of Myb-type transcription factors. Loss-of-function mutations in *ARP* genes induce ectopic *KNOX* gene expression and abnormal leaf development (Waites *et al*. 1998; Tsiantis *et al*. 1999; Timmermans *et al*. 1999; Byrne *et al*. 2000). Misexpression of the *KNOX* genes, *KNAT1* and *KNAT2*, in *Arabidopsis* leaves resulted in transgenic plants with highly lobed leaves. The lobes originated at the serrations of the simple leaf and had features of leaves themselves because of the presence of stipules, suggesting a role for *KNOX* gene regulation in the evolution of leaf diversity (**Fig. 2A, 2B**) (Lincoln *et al*. 1994; Chuck *et al*. 1996). In the compound-leafed tomato, *KNAT1* expression was demonstrated in leaf primordia, in contrast to simple-leafed species, and its overexpression caused supercompoundness with leaves containing up to 2000 leaflets (Hareven *et al*. 1996; Janssen *et al*. 1998). More studies in compound-leafed or dissected-leafed species showed that "ectopic" expression of *KNOX* genes in the leaf primordia occurred naturally (Bharathan *et al*. 2002). In *Cardamine hirsuta* (bittercress), a wild relative of *Arabidopsis*, dissected leaf formation also correlated with the presence of *KNOX* gene activity (Hay and Tsiantis 2006). The conclusion was that naturally occurring *KNOX* gene expression in leaf primordia could impose a state of indeterminacy to cells and could account for dissection, lobing, or compoundness and, to some extent, explain natural variation in leaf shape.

KNOX and plant hormones

In the simple leaves of transgenic lettuce (*Lactuca sativa*) transformed with an overexpression construct of the *Arabidopsis KNAT1*, lobing was observed, in addition to accumulation of specific types of cytokinins (Frugis *et al*. 2001). Resemblance was noticed with phenotypes of plants overexpressing the isopentenyltransferase (*IPT*) gene that encodes an enzyme for cytokinin biosynthesis and with transgenic lines overexpressing *KNOX* (Hewelt *et al*. 1994). Indeed, in *IPT*-overexpressing transgenic lines, ectopic *KNOX* expression was measured (Rupp *et al*. 1999). The question was whether the phenotypic alterations in the *KNAT1* overexpression lines, i.e. the partial reversion to indeterminate growth, are the effect or the cause of cytokinin accumulation (Frugis *et al*. 2001). Today, there are different arguments to say that cytokinin accumulation is a consequence of the induction of cytokinin biosynthesis genes, such as *IPT5* and *IPT7*, by *KNOX* gene overexpression (Jasinski *et al*. 2005; Yanai *et al*. 2005; Sakamoto *et al*. 2006). In other words, the cytokinin accumulation is downstream of the induced *KNOX* gene expression. The question remains whether there is a feedback loop between cytokinin biosynthesis and *KNOX* gene expression.

KNOX gene expression represses gibberellin20 (GA20) oxidases that are involved in GA biosynthesis. So, meristematic activity goes together with low GA content and fits with the view that GA promotes cell differentiation. The antagonistic role of GA was further demonstrated by suppression of the *KNOX* overexpression phenotypes upon addition of external GA that reverted lobing or compoundness back to simple leaf shape (Sakamoto *et al*. 2001; Hay *et al*. 2002). Moreover, high cytokinin levels are necessary and sufficient to stimulate GA2 oxidase, which is involved in GA catabolism, and represents a second mechanism to establish a low GA regime in meristems (Jasinski *et al*. 2005).

Leaf initiation at the SAM occurs through induction of *AS1* gene expression and repression of *KNOX*. In addition, initiation of leaf primordia coincides with local auxin maxima in the SAM (Reinhardt *et al*. 2003). A relationship between the promotion of leaf organ growth by auxin and the leaf fate pathway defined by AS1/KNOX was demonstrated with double mutant analysis and molecular marker lines. Auxin and AS1/KNOX activities are two independent pathways that converge as shown by ectopic *KNAT1/BP* expression in *auxin resistance 1* (*axr1*) mutants, defective in auxin degradation, and by the new leaf phenotype of *as1 axr1* double mutants. Auxin activity is also required later in leaf development to control leaf shape by regulating the initiation of marginal serrations (Hay *et al*. 2006).

LEAFY ectopic expression in pea

In pea, the compound leaf pattern of leaflets and tendrils is gradually formed over a time period of four plastochrons. During this period, form is determined but little growth occurs. At a later stage, the primordium grows at a relatively fast rate, but only in accordance with the organization determined earlier (Sachs 1969). *KNOX* gene activity was not detected in leaf primordia of pea (Hofer *et al*. 2001), in contrast to other compound-leafed species, such as tomato (Hareven *et al*. 1996). Different molecular mechanisms might be at the basis of compound leaf development in pea. Various leaf mutants exist in pea with so-called homeotic phenotypes in which either leaflets were converted to tendrils, such as in the *afila* mutant, or tendrils to leaflets, such as the *tendril-less* mutant, or leaflets and tendrils to a single-leaf lamina, such as the *unifoliata* mutant (**Fig. 2C, 2D**) (Marx 1987). The *UNIFOLIATA* (*UNI*) gene is the homolog of the *FLORICAULA* gene in snapdragon and of the *LEAFY* (*LFY*) gene in *Arabidopsis* that are MADS-box transcription factors specifying floral meristem identity (Coen *et al*. 1990; Weigel *et al*. 1992). Loss-of-function mutation of the *LFY* gene results in the conversion of floral meristems into shoot-like structures. The function of LFY in floral development is to specify determinate growth: if it had the same function in leaves, then more dissected and indeterminate leaves would be expected in loss-of-function mutants. On the contrary, the *uni* mutant reduced leaf dissection. As a consequence, the *UNI/LFY* gene must play role in the lateral leaf organs opposite to that in the lateral flower organs. The role of the *UNI* gene has been postulated to maintain a transient phase of indeterminacy that precedes determination in leaves (Hofer *et al*. 1997).

In conclusion, analysis in several species showed that dissected or compound leaves are a transitional form from the determinate simple leaf to the indeterminate shoot. Acquisition of indeterminacy by the leaf primordia upon initiation and early growth stages is situated in the blastozone with local meristems and results in lamina dissection or leaflet formation. In most species analyzed, this meristematic activity in the leaf primordia coincides with ectopic *KNOX* gene activity and suggests that *KNOX* gene modulation in leaves might be responsible for natural variation in leaf shape. In pea, ectopic activation of *LFY* is correlated with compound leaf formation. Therefore, different molecular pathways might explain the multiple independent origin of dissected leaves during evolution.

LEAF GROWTH DIMENSIONS

Cell division and cell expansion in growth

Leaf growth is a three-dimensional process with proximo-distal, dorso-ventral, and medio-lateral planes and perfect coordination across the dimensions is needed. Each of these dimensions is filled by an intrinsically determined amount of cells that are produced by oriented cell divisions and that expand in a regulated manner. In plants, cell size enlarges by cell growth, total cytoplasmic macromolecular mass increase, and cell expansion, cell volume augmentation through vacuolation. Furthermore, highly polyploid nuclei are associated with increased cell size via enhanced cell

growth (Sugimoto-Shirasu and Roberts 2003; Lee *et al*. 2004). The factors that control the numbers, sites, and planes of divisions and couple these processes to coordinate cell expansion are not well understood (Meyerowitz 1997). Also environmental stimuli regulate growth of an organ; thus, in the meristem perfect coordination is required between developmental controls and growth-regulating mechanisms.

Two theories are used to explain organ formation: the "Cell Theory" that states that organ size and shape are merely determined by their building blocks, the cells and the "Organismal Theory" that proposes that organ shape is predetermined by a higher order control mechanism and that this preset form is filled up with cells (for review, see Tsukaya 2003). A "Neo Cell Theory" adds a level of cellular communication to the original Cell Theory that would allow compensatory effects by, for example, cell enlargement in response to reduced cell numbers (Tsukaya 2002). The Cell Theory implies that individual characteristics of cells determine the morphogenesis of an organ. The simple leaf is a suitable model to investigate this question and has been used in micromanipulation experiments to study the effect of local activation of cell division or expansion on shape (Fleming 2002; Wyrzykowska *et al*. 2002). Transgenic tobacco (*Nicotiana tabacum*) plants were transformed with inducible constructs of either a cyclin gene that triggered cell cycle and, thus, cell divisions, or an expansin gene that stimulated cell wall relaxation and cell expansion. The outcome of local activation of cell division was a leaf lamina with an indentation, while the cell expansion resulted in a lobe. These responses demonstrated the impact of cell growth vs. division status of the cellular building blocks on leaf shape and supported the Cell Theory. The Organismal Theory was originally based on work on green algae in which similar thallus shapes could be observed, irrespective of their organization in syncytia (multinucleate) or single (uninucleate) cells (Kaplan and Hagemann 1991); it separates the three levels of genetic regulation, namely the intrinsic leaf shape, the cell size and shape, and the extent and orientation of cell divisions. As a result, the Organismal Theory states that genetic information specifies the leaf form independently of size and shape of cells and, hence, that leaf size control is at the whole organ level (Green 1976). The Neo Cell Theory links the regulation of cell division and expansion and observes that reduced cell numbers are sometimes partially balanced by increased cell size. Such compensation responses appear to be especially common for misexpression of regulators of cell division (Hemerly *et al*. 1995, 2000; Mizukami and Fisher 2000; Boudolf *et al*. 2004). In many cases, the cell size increase is mediated by polyploidization that results from DNA replication without cell division (Boudolf *et al*. 2004). However, cell expansion to compensate reduced cell numbers can occur not only during cell cycle but also post-mitotically and, therefore, increased ploidy levels are not always required to activate compensation (Ferjani *et al*. 2007). Also environmental conditions, such as shading and moderate soil water deficit, might trigger responses in which the reduced cell numbers associate with an increase in cell size (Aguirrezabal *et al*. 2006; Cookson *et al*. 2006).

The observed complexity in growth and developmental mutants suggests that a higher level coordination of cell division, cell growth, and morphogenesis must exist. Traditionally, mutants and transgenic lines are evaluated based on responses in cell number and size (Tsukaya 2003). However, different growth responses are expected from the misexpression of factors from different regulatory pathways, involving morphogenic transcription factors or regulators of cell cycle, cell growth, or epigenetic control. Furthermore, various organs appear to respond differently because cell expansion rarely occurs in roots to compensate reduced cell numbers (Ferjani *et al*. 2007; Fleury *et al*. 2007). A growth hypothesis has been presented (Fleming 2006) that takes into account the expected phenotypic difference of cell cycle mutants vs. cell growth mutants. Similarly to the yeast models, the cell cycle mutants are predicted to result in reduced number of cells that are large in size, while the cell growth mutants will end up with more, but smaller, cells. Adding another level of complexity, some genes have been shown to regulate the developmental timing of proliferation and expansion during leaf development (Mizukami and Fischer 2000; Autran *et al*. 2002). Reevaluation of the growth theories from the point of view of different classes of mutants might help understanding the growth process and facilitate predicting the outcomes of forward and reverse genetics for application purposes.

Measuring growth parameters

In *Arabidopsis*, the size and shape of leaves vary during the life cycle, a phenomenon called heteroblasty (**Fig. 3A**). Based on morphology (trichome formation on the adaxial and/or abaxial epidermis) and physiology (trichome formation upon addition of GA), rosette leaves are classified as early juvenile (leaves 1 and 2), late juvenile (leaves 3 and 4), and adult (leaves 5 to 7 or 8) (Telfer *et al*. 1997). Cauline leaves are formed after bolting at the inflorescence. Different growth stages of the first leaves have been analyzed histologically and showed gradients of cell division, cell expansion, and cell differentiation from leaf tip to basis and dorsal to ventral side (Pyke *et al*. 1991). Gradients of cell division activity during leaf development have been studied more recently and confirmed with the *pCYCB1;1-GUS* marker gene for mitosis (**Fig. 1C-F**) (Van Lijsebettens and Clarke 1998; Donnelly *et al*. 1999). Obviously, comparison of leaf growth between mutants and wild types would be difficult due to different developmental rate and should be interpreted with caution.

One way to deal with the problem of gradual growth and development is to work on fully expanded leaves, because they can be considered as standardized material in which cell size and cell number are constant and are representative of total cell expansion and cell division that contribute to the final leaf size and shape. For cellular parameter determination, the focus is on one particular leaf at fully expanded stage, either leaves 1 or 2, as in our research group (Nelissen *et al*. 2003, 2005; Fleury *et al*. 2007), on leaf 5 (Tsuge *et al*. 1996), or leaf 6 (Cookson *et al*. 2005) for various reasons. Leaves 1 and 2 allow material to be obtained early in the life cycle, are small in size and easy to manipulate for microscopical or histological analyses. Some research groups prefer leaf 5 or 6, because they correspond to the adult type and originate later in the life cycle, allowing, for instance, measurement of the effect of environmental conditions on their growth (Granier *et al*. 2006). Lamina length, width, and area are analyzed by image analysis on green or cleared leaves (Cnops *et al*. 2004). Cleared leaves serve also for differential interference contrast microscopy to determine leaf growth parameters, such as length, width, and area, in the epidermis (**Fig. 3B**) (Cnops *et al*. 2004). Overall cell numbers (epidermis) can be calculated from the lamina area and cell size (**Fig. 3B**) (Cookson *et al*. 2005). However, cell numbers (palisade) can also be experimentally calculated in serially transverse or longitudinal sections of the leaf (**Fig. 3C**) (Tsuge *et al*. 1996; Nelissen *et al*. 2003; Fleury *et al*. 2007).

Another manner to deal with gradual growth and development is to follow cellular parameters of cell division and expansion in a time course, the so-called kinematic analysis on leaves 1 and 2 (Beemster *et al*. 2005; Fleury *et al*. 2007) or on leaf 6 (Cookson *et al*. 2005). The kinematic growth analysis is often correlated with flow cytometric data in which ploidy levels are measured. In the growing *Arabidopsis* leaves 1 and 2, the epidermis proliferates until day 12 after germination, cells expand until day 19 whereafter cell maturity is reached. The proliferative phase coincides with a high 2C/4C DNA content representative for cell division activity, while endoreduplication starts when cell division rates decline until the end of the cell expansion (Beemster *et al*. 2005).

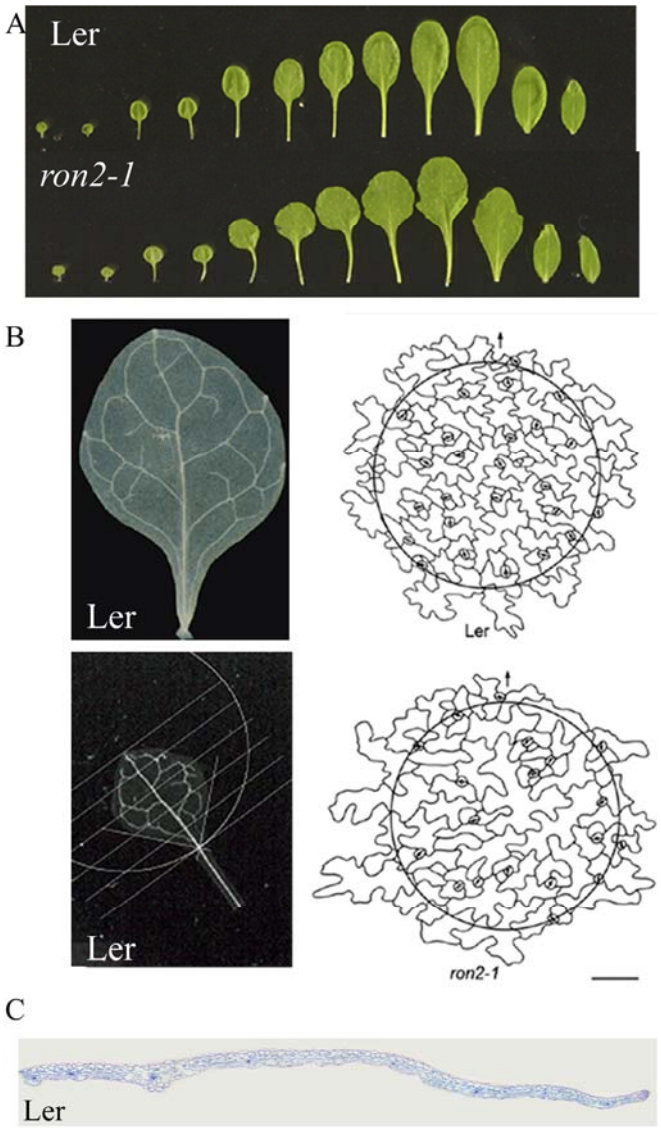

Fig. 3 Measuring leaf growth in *Arabidopsis*. (**A**) Leaf series of *Arabidopsis* wild-type and *ron2-1* mutant (Cnops *et al*. 2004; Nelissen *et al*. 2007). (**B**) *Arabidopsis* leaves cleared from chlorophyll and drawings of upper epidermis visualized from cleared first leaves of wild-type and *ron2-1* mutant plants using differential interference contrast microscopy (Cnops *et al*. 2004; Nelissen *et al*. 2007). (**C**) Transverse sections through an expanded first leaf of an *Arabidopsis* wild type.

Leaf growth plasticity in response to environment

The phenomenon of growth plasticity originates from the ability of plants to acclimate and adapt to different environmental conditions. While the organ shape usually remains unaltered, the size might vary tremendously, because, under unfavorable conditions, plant growth is usually slowed down to allow acclimation processes to take place. Various growth parameters respond differentially and in whole plants, the cell division rate is usually affected by extreme conditions only. At the organ level, the reduction of leaf area is caused by a decrease in cell number per leaf because final leaf area and final cell number are strongly correlated (Dale 1992; Granier *et al*. 2000; Tardieu and Granier 2000; Cookson *et al*. 2005), whereas cell size is reduced, maintained, or increased based on the type of stress (Granier and Tardieu 1999; Cookson *et al*. 2005; Cookson and Granier 2006). Observations of different stress responses suggest that an increase in cell size can be expected when cell division is more affected than tissue expansion, as reported in maize and *Arabidopsis* leaves exposed to water deficit, low phosphorus, or low nitrogen (MacAdam *et al*. 1989; Tardieu *et al*. 2000; Assuero *et al*. 2004) and to low incident light (Cookson and Granier 2006). On the contrary, a decrease in cell size can be expected when tissue expansion is more affected than cell division, as reported for water deficit occurring after cessation of cell division in leaves (Lecoeur *et al*. 1995; Granier and Tardieu 1999; Aguirrezabal *et al*. 2006).

Under salt stress, epidermal cell density is remarkably lower than that of non-stressed plants (Burssens *et al*. 2000). The fast growth responses to stress appear to be mediated directly by cell cycle regulators. The activity of the A-type cyclin-dependent kinase, CDKA;1 has been shown to be highly responsive to environmental cues, such as water deficit (Schuppler *et al*. 1998) and low temperature (Granier *et al*. 2000). Cyclins are essential for CDK activation and have, therefore, been the prime suspects as regulators that couple control of proliferation to the multitude of environmental and developmental pathways that affect growth (Potuschak and Doerner 2001). Promoter activities and transcript levels of the cyclins CYCA2;1 and CYCB1;1 respond fast and transiently and mediate primary growth responses to salt stress (Burssens *et al*. 2000). Cyclins also respond readily to plant growth hormones that might act as links between developmental programs and cell division activity. D-type cyclins have been implicated as direct sensors of environmental mitogenic cues. Application of brassinosteroid hormones is sufficient to induce *CYCD3;1* transcription (Hu *et al*. 2000). GA and abscisic acid (ABA) have been reported to affect G1-to-S progression. In water-submerged rice (*Oryza sativa*) plants, GAs induce expression of *CDKA* and CDK-activating kinase (*CAK*) genes (Lorbiecke and Sauter 1999). ABA inhibits cell division in *Arabidopsis* by decreasing the amount of *CDKA;1* mRNAs and induce expression of the CDK inhibitor gene, *ICK1* (Hemerly *et al*. 1993; Wang *et al*. 1998).

During environmental stresses, growth retardation is caused by fast and sometimes transient cell cycle arrest (Burssens *et al*. 2000). In *Arabidopsis*, shading diminished the relative cell division rate and extended duration of cell division was insufficient to compensate for the decrease in cell division rate, hence the low final cell number. The reduction in epidermal cell numbers is accompanied by an increase in cell size (Cookson and Granier 2006). In *Arabidopsis*, shading also cuts down the number of layers of palisade cells (Kim *et al*. 2005). These changes at the cellular level are reflected in variation of specific leaf weight that was reduced by the shade treatment (Cookson and Granier 2006). In addition to the direct effects on cell division and expansion rates, the developmental timing of transition from proliferation to expansion might also be altered under stress. During normal leaf development, the young leaf primordium is initially in the meristematic state and after a species-specific period of time, the cell proliferation phase is followed by the cell expansion phase. Because the duration of cell division increases by the shading treatment, the *Arabidopsis* leaves act as sink in comparison to those grown under high light intensity. During cell proliferation, the cell growth depends on cytoplasmic growth while the cell expansion is mediated by vacuole expansion. After cell division has ceased, cells expand and the leaves start to photosynthesize and become carbon source (Cookson and Granier 2006).

Somatic polyploidy, due to endoreduplication, is a factor that controls cell size. The cell size of *Arabidopsis* plants decreases under water deficit and is correlated with the reduction in the endoreduplication factor (Cookson *et al*. 2006). However, cell size reduction in plants grown under drought conditions is known to be a ploidy-independent process and to be mediated by diminished cell wall extensibility and/or turgor (Van Volkenburgh and Boyer 1985; Van Volkenburgh 1999). In favor of a ploidy-independent process of cell size control, shade treatment increases cell size despite a decrease in endoreduplication (Cookson *et al*. 2006). Although the environmental conditions studied might have reduced the endoreduplication factor, it might

not necessarily be associated with changes in cell size, but might, to some extent, be due to decreased cell growth, which may be controlled by a low metabolic activity of the cells under stress (Cookson *et al*. 2006).

Reduced leaf expansion and cell division rates have also been observed under water deficit treatments (Granier and Tardieu 1999; Granier *et al*. 2000), whereas the effects of water deficit on the duration of leaf expansion and cell size are not consistent from one study to another (Rawson and Turner 1982; Granier and Tardieu 1999). In *Arabidopsis*, the plasticity of the duration of the expansion phase also affects the plasticity of the final leaf area. The decrease in leaf expansion rate caused by water deficit is, at least partly, compensated by an increase in its duration (Aguirrezabal *et al*. 2006). Proteins and enzymes implicated in the control of the expansion rate and its response to drought stress have been reported (Wu and Cosgrove 2000), but, little attention has been given to the molecular and biochemical events involved (Aguirrezabal *et al*. 2006). Similarly, in *Arabidopsis*, the effect on the rate and duration of leaf expansion is opposite in response to temperature variation (Granier *et al*. 2002) and reduced incident light (Chenu *et al*. 2005; Cookson and Granier 2006). Under water deficit, the plasticity of the final rosette leaf area is caused by that in the area of individual leaves; however, leaf number is slightly affected. Thus, processes of leaf production and expansion are uncoupled to some extent (Aguirrezabal *et al*. 2006). The reason for the relationship between the control of whole-organ and individual cell expansion under different environmental conditions might be the correlation between plasticity of cell size and changes in duration of leaf expansion (Cookson *et al*. 2006).

Plant resistance to fluctuations in environmental conditions is an important agronomical factor. In addition, the plasticity in plant growth and development might be utilized to optimize organ size and shape. However, to fully implement this potential in biotechnology and floriculture, the molecular and genetic regulations of the growth plasticity still calls for better characterization to allow identification of the key regulators.

GENETIC AND EPIGENETIC CONTROL OF LEAF GROWTH

Cell cycle genes

One of the big questions in leaf organ formation is how size and shape are determined. For a long time, cell expansion and its direction have been thought to be the major determinants. More recently, also cell division activity, rate of cell division, and termination of division activity have been found to be important for leaf morphology. Furthermore, the formative cell divisions are considered to be a major factor in morphogenesis. Thus, it is interesting to consider the role of cell cycle in the regulation of plant development in light of transgenic lines that over- or underexpress cell cycle regulatory genes. Mutant approaches have been hard to apply in cell cycle research because mutations in essential cell cycle genes are often obscured by redundancy (Thomas 1993; Himanen *et al*. 2003) or the phenotypes are too severe and might provoke embryonic lethality. Effects on growth might also arise from mutations in metabolic pathways and are difficult to distinguish from those directly related to cell division control (Traas and Laufs 1998).

CDKA;1 is a key regulator of the plant cell cycle, it is constitutively produced, but its kinase activity is regulated by (de)phosphorylations and interactions with regulatory subunits, such as cyclins, CDK subunits (CKS) and inhibitors (CKI). The expression of a dominant negative mutation in CDKA;1 under the cauliflower mosaic virus 35S promoter (*CaMV35S-cdc2a.N147*) is embryo lethal in *Arabidopsis* and only when expressed under an embryo-specific promoter of the albumin gene *2S2*, embryos, albeit distorted, were formed (Hemerly *et al*. 1995, 2000). In tobacco, expression of the heterologous *CaMV35S-cdc2a.N147* inhibited cell division activities in the transgenic plants, resulting in normally differentiated leaves with fewer, but enlarged cells (Hemerly *et al*. 1995). In *Arabidopsis*, overexpression of *CKS* reduces leaf size and root growth rates, caused by increased cell cycle duration and reduced meristems (De Veylder *et al*. 2001a); overexpression of *CAK* diminishes CDK activity and early differentiation of the *Arabidopsis* root initial cells (Umeda *et al*. 2000); and overexpression of the *Arabidopsis Kip related protein 1* (*KRP1*) and *KRP2* genes strongly inhibit mitotic cell divisions and cause serrated leaf morphology with increased cell size (Wang *et al*. 2000; De Veylder *et al*. 2001b). In all these cases, cell division activity is reduced because of impaired CDKA;1 activity and, in at least some cases, has early differentiation as a consequence.

D-type cyclins are mitogen responsive, promoting cell cycle activation at the G1-to-S transition together with CDKA;1. A number of D-type cyclins have been ectopically expressed in plants, allowing comparison of their phenotypes with those of the experiments with CDKA;1. Ectopic expression of *CYCD3;1* reduces cell size and alters cell cycle duration. In developing and mature leaves, the total cell number is increased, but cells are unable to fully differentiate (Dewitte *et al*. 2003). However, the ectopically induced cells in the leaves acquire the correct cell identity. The transcription factor *AINTEGUMENTA* with APETALA2 domain regulates the number of cells incorporated into developing leaves. Its overexpression causes extra cell divisions in association with high *CYCD3;1* expression, suggesting that CYCD3;1 could also function at mitosis (Schnittger *et al*. 2003). Driving cells over the G1-to-S transition might also commit these cells irreversibly to mitosis. Overexpression of *CYCD2;1* in *Arabidopsis* promotes cell production through enhanced CDK activity and shortening of the G1 phase duration (Qi and John 2007). Moreover, the increased cell numbers do not result in enhanced organ growth because the cell expansion is inhibited. Thus, the final cell size appears to be controlled at the levels of cell cycle and tissue rather than by cell-autonomous control of expansion (Qi and John 2007). E2F/DP transcription factors act downstream of D-type cyclins, but ectopic expression of *E2F* alone only increases the cell number in the cotyledons. The combination of overexpression of the dimer-forming E2F/DP transcription factors triggers cells to divide ectopically during leaf development and to inhibit differentiation (De Veylder *et al*. 2002). The mitotic B-type cyclins promote the G2-to-M transition of the cell cycle. In the *reduced CYCB1;1 expression* (*rcb*) mutant, the expression of the mitotic *CYCB1;1* is abolished from the *Arabidopsis* shoot and root meristems (Himanen *et al*. 2003), but, no severe growth defects are observed, except in the inflorescence stems.

Unlike the growth rate and cell numbers, pattern formation appears to be regulated outside from the core cell cycle and rather to be linked at the organismal level. However, plant morphogenesis is determined by the oriented cell division and cell expansion patterns. The plane of cell division is involved in the direction of cell expansion and is, thus, an important morphogenetic factor. In plant cells, the cell division plane is specified during the G2 phase by assembly of the preprophase band at the site of future cell plate formation. Although the mechanism is unknown, activities of cytoskeleton and vesicle transport are seemingly comprised (Torres Ruiz and Jürgens 1994). Developmental cell divisions are often asymmetric and the orientation of the cell division as well as the cell fate is determined by positional cues (Ruth *et al*. 1985; van den Berg *et al*. 1995).

Endoreduplication is an alternative mode of the cell cycle in which DNA replication is not followed by nuclear and cellular division, resulting in polyploidization of the cell. A positive correlation between nuclear DNA content and volume has been observed in epidermal cells of young leaves of both endopolyploid and non-endopolyploid species. Moreover, nuclear size and DNA content are positively correlated and mature cell volume of epidermal cells with-

out vacuoles does not depend on the endopolyploidization state of the species (Melargano *et al*. 1993; Jovtchev *et al*. 2006). The alteration of the endocycle is associated with changes in final cell and organ sizes and supports the cell theory of organ size control (Sugimoto-Shirasu *et al*. 2002; Castellano *et al*. 2004).

Taken together, modifications in cell cycle machinery might provide opportunities to affect the growth rates, cell numbers, and their size. Because endoreduplication cycles might contribute to a faster growth in endopolyploid than in non-endopolyploid species of the same basic genome size, thereby modifying the ploidy levels, identification of the regulators might provide opportunities for optimizing many aspects of crop performance.

Forward and reverse genetics

The intrinsic leaf size is determined by the number of cells produced by cell division activities during the early stages of primordium formation (Mizukami and Fischer 2000). The core cell cycle machinery is regulated by upstream developmental and environmental cues. Key components of these signaling pathways need to be identified to understand the molecular mechanisms that control leaf size by regulating cell number. The main approach so far has been mutational analysis or so-called forward genetics, in which mutations are induced either by chemicals, radiation or heterologous DNA, such as transposons or the *Agrobacterium tumefaciens* T-DNA. The corresponding genes are cloned either by fine mapping and candidate gene approach, designated map-based cloning or by polymerase chain reaction methods that make use of the known DNA sequence of the heterologous DNA, designated gene tagging (**Fig. 4**). For example, the collection of EMS-induced leaf mutants in *Arabidopsis*, representing 94 gene loci (**Fig. 5A-S**) (Berná *et al*. 1999), is an important resource for gene identification in leaf growth ad development and several genes have been cloned today (Pérez-Pérez *et al*. 2002, 2004; Cnops *et al*. 2004; Nelissen *et al*. 2005; Hricová *et al*. 2006; Fleury *et al*. 2007).

A number of transcription factors and transcriptional co-activators have been reported to affect leaf shape and size by regulating cell numbers (Mizukami and Fischer 2000; Kim *et al*. 2003; Kim and Kende 2004; Horiguchi *et al*. 2005). The leaf lamina width of the respective knockdown mutants is reduced, whereas that of overexpression lines is increased. So far, no link to core cell cycle regulation has been studied or demonstrated for these transcription factors. In addition, interference with ribosome biogenesis and, hence, cellular growth also affects cell number in leaves (Van Lijsebettens *et al*. 1994; Ito *et al*. 2000). New signaling-type proteins have been identified and found to act in the same genetic pathway, i.e. TRN1, encoding a LRR-type protein without kinase domain, and TRN2, a tetraspanin protein containing membrane spanning domains. These proteins affect cell numbers in the leaf lamina, lamina symmetry, and venation patterning and, hence, coordinate early patterning events during leaf growth (Cnops *et al*. 2006). The *TRN2* gene has a function in the peripheral zone of the SAM where it controls cell numbers (Chiu *et al*. 2007). Genes have been cloned that influence leaf size by regulating cell expansion. One of the expansin genes has been overexpressed to modulate cell wall extensibility and has resulted in increased leaf size (Cho and Cosgrove 2000). Knockdown mutants in the *ANGUSTI-FOLIA* (*AN*) gene, coding for a CtBP transcriptional co-repressor, resulted in reduced leaf width because of decreased polar cell expansion as a consequence of abnormal arrangement of cortical microtubuli (Folkers *et al*. 2002; Kim *et al*. 2002). Polar cell expansion in the leaf length direction is also influenced by the *ROTUNDIFOLIA3* (*ROT3*) gene that codes for a cytochrome P450 steroid hydroxylase with a steroid substrate recognition site and might be involved in steroid biosynthesis (Kim *et al*. 1998). The pathways for polar cell expansion in the width and length directions, identified by the *an* and *rot3* mutations, respectively, are independent (Tsuge *et al*. 1996). In the meantime, a new collection of leaf mutants has been described and their growth defects in terms of cell division and expansion are being characterized (Horiguchi *et al*. 2006).

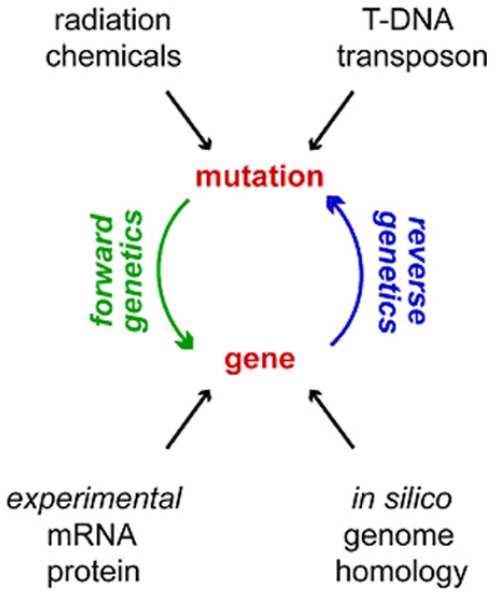

Fig. 4 Forward and reverse genetics. Forward genetics starts from a mutation, induced by radiation, chemicals, or heterologous DNA insertions (T-DNA and transposons) to clone the corresponding gene. For reverse genetics, the starting point is the known gene sequence, derived from experimental mRNA or protein sequence, from the genome sequence (*in silico*), or through homology with other organisms to look for the corresponding mutant in existing collections.

In the future, reverse genetics will become more important to study leaf organ formation: starting from genes identified *in silico* from the genome sequence, differentially expressed in microarray analyses (Beemster *et al*. 2005) through homology with other organisms (Nelissen *et al*. 2005), or identified by protein analyses (**Fig. 4**). These genes, functionally characterized by investigating their corresponding mutants, can be purchased from the stock centers. Indeed, today, insertion alleles are accessible for nearly every gene in the *Arabidopsis* genome and can be looked for on the website of The Arabidopsis Information Resource (TAIR) (http://www.arabidopsis.org/index.jsp), and requested.

Epigenetic control

Chromatin modification results either in transcription activation or repression and might act upstream of transcription factors and the core cell cycle. There is also accumulating evidence that chromatin is reactive to environmental stimuli, such as light (Offermann *et al*. 2006) and, hence, might operate as an interface that receives signals from the environment and transduces them into transcriptional activity (for review, see Nelissen *et al*. 2007). One of the intriguing questions in chromatin biology is to what extent the chromatin-modifying complexes are gene specific.

From several leaf mutants of the EMS collection (Berná *et al*. 1999), genes have been identified with a function in chromatin modification. The four *ELONGATA* (*ELO*) loci (**Fig. 5**) correspond to Elongator, a histone acetyl transferase (HAT) complex with a conserved subunit structure comparable to that of yeast (Nelissen *et al*. 2005). In yeast, Elongator has been shown to associate with the RNA polymerase II (RNAPII) transcription elongation complex. HAT activity results in the acetylation of histone H3 in the nucleosomes and is postulated to make the DNA more accessible for transcription through RNAPII (Otero *et al*. 1999; Hawkes *et al*. 2002). In plants, we have shown that the

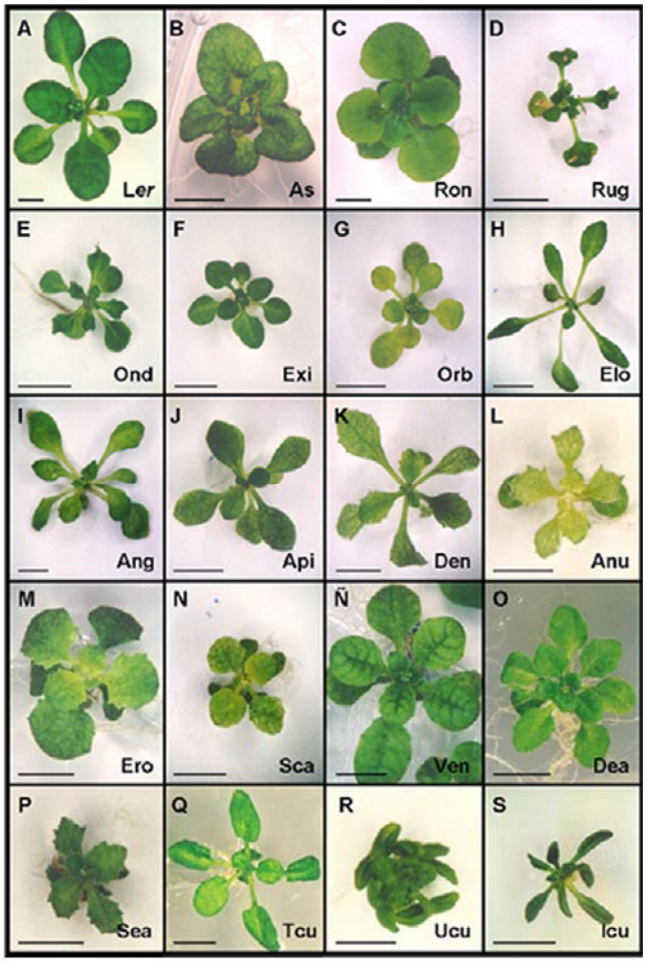

Fig. 5 ***Arabidopsis*** **(L.) Heynh. leaf mutant classes of L*er* ecotype from an EMS-mutagenized collection.** (kindly provided by Prof. J.L. Micol).

Elongator complex has a conserved structure and has a function in leaf and root growth by regulating the cell numbers. Indeed, knockdown mutants in the *ELO* genes form narrow leaves (**Fig. 5H**) with reduced root growth (Nelissen *et al*. 2005). The *elo4* mutation is allelic to the *drl1-2* mutation as predicted also from the similar narrow-leaf phenotype. The *DRL1* gene is the homolog of the yeast KTI12/TOT4 gene that is a putative regulator of Elongator (Nelissen *et al*. 2003). The *ELO2* gene, encoding the largest subunit of the Elongator complex, modulates ABA and drought responses in plants, indicating a role for Elongator in sensing the environment and adjusting plant growth and development to adverse conditions (Chen *et al*. 2006). Elongator controls cell proliferation through metabolism rather than action on the core cell cycle machinery. Indeed, the *elo* mutants have unaltered flow cytometry profiles (Nelissen *et al*. 2005), but a different growth response when the sucrose concentrations are increased in the medium and the sugar metabolism-related genes have a genotype-dependent expression (Falcone *et al*. 2007).

Another mutant, *hub1-1* (previously designated *ang4;* Berná *et al*. 1999), is a narrow-leaf mutant (**Fig. 5I**) with decreased cell numbers in both leaf epidermis and palisade cell layers (Fleury *et al*. 2007). The mutant has a severely reduced cell division rate during the early stages of leaf development because of a block at the G2-to-M transition and, therefore, a prolonged cell cycle duration. In *hub1-1*, the timing of cell proliferation and growth is not altered during the leaf development and the leaf growth ceases 18 days after sowing, similarly as for the wild type, thus before the intrinsic organ size has been reached. Although irregular cell sizes have been observed in the palisade parenchyma of the leaf sections, the average cell size in the leaf epidermis remains unchanged when compared to the wild type. Endoreduplication levels are increased in the leaves, which might explain that the epidermal cell size is not reduced. Instead, in the roots, the average cell length is reduced by 50%, suggesting that HUB1 might also govern cell growth. Molecular characterization of *HUB1* has revealed a role in histone H2B monoubiquitination that has been associated with chromatin activation and transcriptional programming through RNAPII in other organisms (Wood *et al*. 2003; Zhu *et al*. 2005; Fleury *et al*. 2007). In contrast to single transcription factors that control specific growth parameters during leaf growth (Mizukami and Fischer 2000; Horiguchi *et al*. 2005), HUB1 might be a novel upstream regulator of organ growth and development.

In summary, we have identified two conserved histone-modifying complexes in plants that control leaf and root growth by cell proliferation and postulate that their intrinsic activity contributes to leaf size and shape that are species specific. In addition, the histone-modifying complexes might act as an interface between the environment and RNAPII to adjust transcription, hence contributing to plasticity in leaf formation.

FUTURE PERSPECTIVES AND APPLICATIONS

Unraveling the complex regulatory networks of leaf initiation from SAM and leaf growth has pointed out intricate transcriptional interactions that orchestrate the developmental decisions (Byrne 2005). Cell division and expansion are regulated downstream of the transcriptional network to provide the actual building blocks of the organs. Much less explored and understood processes are those underlying the actual growth related to biosynthetic components that are required for both meristem-associated and vacuole-associated growth (Chen *et al*. 2001; Fleming 2006). Such processes could represent transcriptional programming, ribosomal biogenesis, and other metabolic pathways (Van Lijsebettens *et al*. 1994; Fleury *et al*. 2007). Furthermore, epigenetic control appears to emerge as a higher upstream level to coordinate cell division and growth during organ development (Nelissen *et al*. 2005; Fleury *et al*. 2007).

Leaves are the sites of primary production in plants; indeed, by the process of photosynthesis, atmospheric CO_2 is converted into organic compounds that are the basis for more complex molecules. Leaves are shaped as flat organs to maximize light capture that is influenced by phyllotaxis, leaf emergence rate, leaf size and shape, and also affects biomass production. So, developmental genes with a function in either of these leaf parameters could be of interest in crops and vegetables to increase biomass or even seed yield. In addition, variation in leaf shape might be of commercial value in ornamentals and horticultural varieties, such as lettuce, in which area, size, and color are traits of interest. The genes that control leaf serration and compound leaf formation might have an impact on this market in the future. The vegetable growing and supply industry is an intensive, innovative, high-value agricultural sector. To provision a large range of markets an extensive portfolio of products is essential. For various growing areas, growth seasons, and market segments and destinations, specific and sophisticated vegetable hybrids and varieties are needed. Currently, companies specialized in production of vegetable varieties for the professional growers focus on developing varieties that create value through increased yield, insect and disease resistances, and improved qualities, such as taste, color, size, and longer shelf and field life (http://www.nunhems.com/default.asp).

As fossil fuel is running out of supply, the need for alternative types of energy is becoming central. The conversion of solar energy by plants into green biomass or wood, and seed yield are the basis for bioethanol and biodiesel, respectively, and provides an important alternative to fossil fuel. Therefore, genes that determine aspects of plant production, i.e. leaf size, phyllotaxis, wood quality, oil composition, will be explored in the near future to improve quality of energy crops, such as the C4 grasses *Miscanthus* or *Sorg-*

hum bicolor (sorghum) and the poplar (*Populus* species) trees. Transgene technology or marker-assisted breeding might be used to change traits in plants and be more generally applicable for modification of qualitative traits in commercial varieties because it only requires one gene of interest, its cloning into a suitable plant transformation vector, and its introduction into the plant (Van Camp 2005). However, especially for food and fodder purposes, a general consensus is still lacking that genetically modified organisms are safe and acceptable. In ornamentals and fiber crops, such as cotton (*Gossypium hirsutum*), a genetical modification approach could be more acceptable as the preferred way to proceed. An alternative method is the marker-assisted breeding in which promising endogenous alleles are followed in breeding programs by using molecular markers to trace them and to speed up the selection procedure. In any case, the two mentioned technologies have the potential to generate crops and varieties that are better suited for their production process and adapted to the needs of market and consumer.

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