Size Control in Plants—Lessons from Leaves and Flowers

Hjördis Czesnick and Michael Lenhard

Institute for Biochemistry and Biology, University of Potsdam, Potsdam 14476, Germany

Correspondence: mlenhard@uni-potsdam.de

To achieve optimal functionality, plant organs like leaves and petals have to grow to a certain size. Beginning with a limited number of undifferentiated cells, the final size of an organ is attained by a complex interplay of cell proliferation and subsequent cell expansion. Regulatory mechanisms that integrate intrinsic growth signals and environmental cues are required to enable optimal leaf and flower development. This review focuses on plant-specific principles of growth reaching from the cellular to the organ level. The currently known genetic pathways underlying these principles are summarized and network connections are highlighted. Putative non-cell autonomously acting mechanisms that might coordinate plant-cell growth are discussed.

Over millions of years, plant leaves and flowers evolved into an enormous range of shapes and sizes. Likely reflecting adaptations to changing environmental conditions, even closely related species often differ dramatically in their organ sizes (Mizukami 2001). Although interspecies diversity is remarkably high, species-specific leaf and petal characteristics are often highly uniform between individuals grown under constant conditions. This suggests that tight genetic control is used to integrate intrinsic growth signals and environmental cues to enable organ growth to a defined size. This review summarizes the current knowledge of the regulatory networks of plant size control at the cellular and at the organ level. We will focus on the regulation of determinate growth of lateral plant organs, such as simple leaves and petals.

INITIATION AND DEVELOPMENT OF LEAF PRIMORDIA

Developmental patterning begins in the growing embryo, where the plant's radial structure and the apicobasal axis are established (Steeves and Sussex 1989). Later, one or more stems with repeatedly emerging leaves grow out and, eventually, flowers will develop from the shoot apical meristem (SAM).

Leaves are essential organs to ensure photosynthesis and carbon assimilation, and are being iteratively generated by the growing plant. In contrast to animal bodies, plant stems have the potential to grow indeterminately. In higher plants, lateral organs are initiated by the SAM located at the apical pole of the plant. The SAM consists of pluripotent cells that are functionally equivalent to stem cells in animals (Aichinger

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et al. 2012). Meristems can maintain themselves throughout the plant's lifespan while continuously providing cells that can enter differentiation pathways. Recently, Murray et al. (2012) reviewed the initiation of leaves at the SAM.

A leaf primordium is initially defined by a local auxin maximum at the flanking region of the SAM (Reinhardt et al. 2003). Soon after, leaf dorsiventrality is established and a marginal meristem develops (reviewed in Tsukaya 2013). To allow outgrowth of the primordium, the rigid cell walls at the initiation site have to be loosened and elasticity is increased by pectin modifications (Peaucelle et al. 2011). The founder cells incorporated into the primordium first proliferate by coordinated cell division, which may involve species-specific orientation patterns (Dolan and Poethig 1998). In concert with cell division, the cytoplasmic mass grows continuously, while the overall cell size remains largely constant. The proliferation phase is then followed by a cell-expansion phase. Expansion is mainly driven by cell-wall modifications accompanied by increasing turgor pressure because of water uptake into the vacuole (Schopfer 2006). Endoreduplication, the increase of ploidy by chromosome replication without subsequent cell division, is also often involved in the process of increasing cell size. Many basic questions on how final organ size is achieved are still not understood completely. Does an organ measure its own size and, if so, how? Eventually, the total cell number has to be in accordance with the final organ size. But when and how do individual cells know that proliferation has to cease? Different aspects considering cell proliferation and its arrest are described in the literature. Thereafter, mechanisms of determining cell size as well as the consequences of having to coordinate growth at the organ level will be discussed.

SIZE INCREASE BY PROLIFERATIVE GROWTH

The final size of a leaf is critically influenced by the duration of cell proliferation. Initially, the primordium cell number increases through division. To ensure an appropriate final organ size,

a timely transition to postmitotic cell expansion is essential. Observations of cell-cycling patterns in *Arabidopsis thaliana* and *Antirrhinum majus* (hereafter called *Arabidopsis* and *Antirrhinum*) leaves revealed that division begins to arrest at the distal tip (Donnelly et al. 1999). Then a so-called cell-cycle arrest front moves toward the leaf base (Fig. 1) (Nath et al. 2003). Recent studies indicate that the boundary between proliferating and nonproliferating cells appears rapidly, and is maintained at a constant distance from the leaf base for several days. Proliferation of all cells then stops rapidly again, indicating that the process does not happen as gradually as expected before (Kazama et al. 2010; Andrianakaja et al. 2012). Proliferation of dispersed meristematic cells (DMCs) forming stomata and the vasculature continues for longer, before it is terminated by a second arrest front.

How to Measure the Size of a Growing Leaf?

The cell number that is set during the proliferative growth phase is crucial for the final organ size. But how does an organ sense that it will reach the correct size if cell cycling is terminated?

One model that aims to explain the process of proliferation arrest suggests that the growing primordium measures its own size or the size of the proliferative region. A gradient of an extracellular proliferation-inducing signal, which is produced at the base of the leaf, could serve as a size indicator. This strategy has been suggested for *Drosophila* wing size determination (Hufnagel et al. 2007). A morphogen is synthesized in the center of the growing wing. When a certain size has been reached the cells at the organ periphery cease to proliferate owing to a lack of the growth factor. The mechanical stress resulting from ongoing growth of the more central cells versus the growth arrest in the periphery is then thought to induce the termination of cell division throughout the wing. A similar scenario has been discussed for the plant leaf, with the putative growth signal produced by the cytochrome P450 KLUH/CYP78A5 in the organ periphery being progressively diluted in the growing organ (Anastasiou et al. 2007; Kazama

Figure 1. Main genetic factors controlling lateral organ growth in plants. The final size of a leaf is determined by the rate and duration of cell proliferation and the extent of postmitotic cell expansion. Both growth phases are controlled by a number of growth-promoting and growth-inhibiting factors. Cell division arrests first at the leaf tip, followed by a cell-cycle arrest front moving toward the leaf base. Proliferating cells are indicated by small squares, whereas expanding cells are presented as larger squares. The arrest front is indicated by the bright green border between the proliferation and the expansion zone. Not indicated are the factors PEAPOD1/PEAPOD2, which inhibit the proliferation of dispersed meristematic cells, and thus support the progression of a secondary arrest front. The following factors have not been discussed in detail in the main text: Rotundifolia4 (ROT4) (Ikeuchi et al. 2011), Ngatha (NGA) (Alvarez et al. 2009), Spatula (SPT) (Ichihashi et al. 2010), Struwwelpeter (SWP) (Autran et al. 2002), ARGOS-LIKE (ARL) (Hu et al. 2006), and CYP78A7 (a homolog of KLUH/CYP78A5) (Wang et al. 2008). TOR, Target of rapamycin; RAPTOR, regulatory-associated protein of TOR; GRF, growth-regulating factor; GIF, GRF interacting factor.

et al. 2010). However, later clonal analysis has indicated that the range of activity of the *KLUH*-dependent signal extends beyond individual organs, making this scenario less likely.

Another model assumes that the growing primordium measures its size indirectly by counting the number of cell divisions. Again

a molecule (e.g., a cell-cycling inhibitor or promoter), could accumulate or be degraded continuously with the cell-division progress. Such an intracellular growth-sensing mechanism could explain why many animal precursor cells stop dividing after a certain number of times (Conlon and Raff 1999). However, to

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our knowledge, no convincing candidate for such a factor has been described in plants. Assuming a limited number of cell cycles, final organ size could also be influenced by the initial number of cells recruited from the SAM to the emerging primordium. The primary size of the founder cell cluster varies between plant species (Dolan and Poethig 1998), and for one petal-size and two leaf-size mutants of *Arabidopsis*, an altered primordium size could be shown to correlate with changes in final organ size (Autran et al. 2002; Eloy et al. 2012; Trost et al. 2014).

A third model assumes that the duration of the cell proliferation period is fixed, allowing cells to proliferate only for a limited amount of time. Under this scenario, changes in the rate of cell production should modify organ size. Indeed, when the rate of cell production is altered because of either a change in the pace of cell cycling or of the size of the proliferative area at the base of the leaf, corresponding changes in organ size are seen (Ferjani et al. 2007; Lee et al. 2009; Rojas et al. 2009; Ichihashi et al. 2010; Ikeuchi et al. 2011). A number of mutants are known that prolong or reduce the duration of the proliferation phase (see below). Yet, how they determine this duration and whether they interact with a size-sensing mechanism is not well understood.

The above discussion also touches on the question of whether organ growth in plants should rather be viewed as a cellular or a supra-cellular process, with control acting at the organ-wide level. Defects in cellular processes, such as division or cytoplasmic growth, clearly do result in altered organ sizes (see below). However, several observations also suggest the involvement of regulation above the cell level. These include the classical findings by Foard and Haber (1961) that wheat leaves can grow to a normal size and shape even when cell division is severely impaired, the phenomenon of compensation, and the identification of several non-cell-autonomous factors that influence organ size (see below).

Taken together, the growing leaf could use different parameters to estimate and keep track of its own size. The idea of directly measuring the target parameter, which is the current pri-

mordium size, is particularly appealing because it is robust and integrates fluctuations of cell-division patterns. However, how this would be achieved molecularly is far from understood.

Principles of Proliferation Control: Genetic Evidence

In recent years, regulatory pathways controlling the arrest of cell division have been elucidated in more detail. A summary of the genetic pathways discussed here is provided in Figure 1. The analysis of mutants with altered cell numbers led to the discovery of diverse proliferation promoting as well as inhibiting factors in *Arabidopsis* and *Antirrhinum* (Fig. 2). Because cell size and cell proliferation are intimately linked, a number of regulatory elements influence both processes.

Genetic pathways regulating growth are often activated by phytohormones. The expression of the small plant-specific protein ARGOS is strongly induced by auxin (Hu et al. 2003). ARGOS is ER-localized and promotes cell proliferation by stimulating the expression of the DNA-binding protein AINTEGUMENTA (ANT), which, in turn, up-regulates a cell-cycle activator, the D-type cyclin CYCD3;1 (Mizukami and Fischer 2000). Mutants of ARGOS and ANT possess smaller leaves with a reduced number of cells because of a premature proliferation arrest. The high sequence similarity of the DNA-binding domain of ANT and AINTEGUMENTA-LIKE (AIL) proteins led to the suggestion that these proteins might act redundantly (Krizek 2009). However, AILs might have distinct and even opposite functions during meristem development (Mudunkothge and Krizek 2012). The proliferation promoting effects of ANT can be repressed by a member of the auxin response factor family, ARF2. This hormone-responsive factor inhibits the activity of ANT and CYCD3;1 and can itself be inactivated by phosphorylation through the brassinosteroid-responsive kinase BIN2 (Schruff et al. 2006; Vert et al. 2008), providing a possible point of integration for auxin and brassinosteroid signaling.

A second important pathway regulating the timing of proliferation is a cascade of tran-

Figure 2. Phenotypic alterations of size regulator mutants. Mutant phenotypes of determinate plant organs that are based on defects in cell proliferation or cell expansion are shown schematically. The final organ size can be altered because of changes in cell numbers or cell sizes, exemplified by squares in the simplified organ. Factors that are involved in the inhibition or promotion of cell proliferation, and factors involved in the promotion or inhibition of cell expansion (from left to right) are listed below the individual mutant leaves.

scription factors and microRNAs (miRNAs), consisting of the TEOSINTE BRANCHED1/CYCLOIDEA/PCF transcription factor family (TCP), which targets growth-related genes and is, in turn, targeted by miRNAs. Based on sequence homologies, the 24 TCP transcription factors encoded by the *Arabidopsis* genome can be divided into two classes. Class II TCPs, which are also referred to as CIN-TCPs, are essential for timely proliferation arrest in leaves by promoting cell differentiation (Efroni et al. 2008). In addition, the class II factor TCP4 restricts proliferative growth indirectly by damp-

ening responses to cytokinin, which itself has a stimulating effect on mitotic cell division (Fig. 3) (Miller et al. 1955; Efroni et al. 2013). The *Antirrhinum* mutant *cincinnata* (*cin*) has a defect in a class II TCP and produces crinkly leaves owing to prolonged cell proliferation (Nath et al. 2003). A similar phenotype is shown by the *Arabidopsis* *jaw-D* mutant (Palatnik et al. 2003). The *jaw-D* mutant overexpresses miR319a, a miRNA that targets and represses the activity of five members of the TCP family. Accordingly, plants expressing miR319-resistant TCPs show smaller organs because of early

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Figure 3. CIN-TCPs regulate the *Arabidopsis* leaf response to cytokinin. The mode of action of class II TCPs is illustrated by images of 21-day-old plants expressing the cytokinin biosynthesis enzyme isopentenyl transferase (*IPT*) or the cytokinin (CK) deactivating enzyme *CKX3* from the *BLS*-promoter, which is active in young leaves. Increased CK levels lead to the formation of small yellow leaves with excessive serrations, whereas the reduction of leaf CK leads to the formation of smaller rounder leaves. If *miR319* is overexpressed in the *BLS>IPT* background, leaves are even smaller and never mature. The excessive leaf serrations caused by the overexpression of *miR319* can be eliminated by decreased CK levels, demonstrating that *miR319* acts through CK. If a *miR319*-resistant form of *TCP4* (*rTCP4*) is expressed in the leaves, the whole shoot shows an early growth arrest. The alteration of CK levels has little effect in the *rTCP4*-overexpressing background. WT, wild type; (From Efroni et al. 2013; reprinted, with permission, from Elsevier © 2013.)

cell differentiation (Ori et al. 2007; Nag et al. 2009). Class II TCPs, in turn, stimulate the expression of *miR396*. *miR396* targets mRNAs encoding several members of the proliferation-promoting growth-regulating factor (GRF) transcription factor family (Liu et al. 2009; Rodriguez et al. 2010). In concert with a family of transcriptional coactivators termed GRF-interacting factor (GIF), GRFs maintain cell division and mutants with defective GRFs or GIFs show smaller, narrower leaves (Kim and Kende 2004; Horiguchi et al. 2005; Lee et al. 2009). Several factors of this pathway, like *CIN-TCPs* or *miR396* are expressed in a spatiotemporally controlled manner in the leaf (e.g., distal or proximal to the proliferation arrest front) (Nath et al. 2003; Wang et al. 2011). Thus, antagonistically acting processes in the expansion

and proliferation zones have been suggested to regulate the progression of the cell-division arrest (Andriankaja et al. 2012).

Based on results for the class I TCP-family member *TCP20*, the group of class I TCPs had been suggested to support proliferative growth in contrast to the growth-restricting class II TCPs (Li et al. 2005). Recently, this classification has been challenged as class I TCPs 14 and 15 modulate cell proliferation and expansion in an organ and cell context-dependent fashion (Hervé et al. 2009; Kieffer et al. 2011). Interestingly, the DNA-binding activity of *TCP15* can be reduced by oxidation of a cysteine residue typical for class I TCPs (Viola et al. 2013). Thus, TCPs might modulate leaf growth and shape under stressful conditions in response to changes of the cellular redox homeostasis.

A different strategy to fine-tune growth processes is the targeted decay of key factors by proteasomal degradation. The ubiquitin binding protein DA1 (from the Chinese word DA meaning “large”) acts synergistically with the E3 ubiquitin ligase DA2 to restrict organ and seed size (Li et al. 2008; Xia et al. 2013). The *da1-1* and *da2-1* mutants generate large leaves attributable to prolonged cell proliferation with double mutant leaves being even larger. Similarly, the E3 ligase BIG BROTHER (BB) also limits the proliferative-growth phase, again acting synergistically with *da1* (Disch et al. 2006; Li et al. 2008).

A cell-autonomously acting factor stabilized by auxin is EBP1, the ErbB-3 epidermal growth factor receptor-binding protein. EBP1 supports proliferation by activation of D- and B-type cyclins and in parallel limits the size of proliferating cells (Horváth et al. 2006). Human EBP1 is suggested to be involved in ribosome biogenesis (Squatrito et al. 2004). The high interspecies protein sequence similarity indicates a similar function in plants.

With the differentiation of leaf cells the photosynthetic apparatus develops in the chloroplasts, transforming leaves from sink into source organs. In accordance, genes involved in cell-cycle regulation, photosynthesis, and chloroplast retrograde signaling are differentially expressed in proliferating compared to expanding cells. In fact, signals released from the maturing chloroplast have been suggested to support the onset of cell expansion and, thus, proliferation arrest (Andrianakaja et al. 2012). An additional link between cellular growth and carbon metabolism, which is influenced by environmental conditions, is provided by the signaling molecule trehalose-6-phosphate (reviewed in Schluepmann et al. 2012).

The DMCs like stomatal and vascular precursors terminate their division later than the bulk of lamina and epidermis cells (White 2006; Andrianakaja et al. 2012). Considering that guard cells constitute up to 30% of all leaf epidermis cells and stomatal lineages generate up to 82% of the epidermis cells, it is obvious that the controlled division of DMCs makes an important contribution to final organ size (Geisler

et al. 2000). The progression of a secondary basipetal division-arrest front affecting DMCs is controlled by two DNA-binding proteins: PEAPOD1 (PPD1) and PPD2. A *ppd1 ppd2* mutant shows a prolonged DMC proliferation phase and, thus, excessive lamina growth particularly in the center, resulting in a dome-shaped leaf (White 2006).

REGULATION OF CELL SIZE

Cell proliferation is inseparably linked to cell growth. Early during leaf development, an appropriate cell size is critical for the commitment to cell division and may provide a checkpoint for cell cycle control (Francis and Halford 1995; Donnelly et al. 1999). Later, when the leaf blades of tobacco or *Arabidopsis* have reached ~10% of their final size, mitotic cell divisions begin to arrest in favor of eventual cell expansion (Poe-thig and Sussex 1985; Donnelly et al. 1999; Efroni et al. 2010). The final cell size and concomitant processes, such as the cytoplasmic mass increase, turgor adjustments, and cell-wall modifications, are regulated depending on the tissue layer. In the following, the regulation of cell expansion at different levels will be discussed. The extensive roles of phytohormones in modulating cell expansion have been reviewed elsewhere (Wolters and Jürgens 2009; Vanstraelen and Benková 2012).

Growth Control at the Level of Translation

To maintain an appropriate cell size through successive rounds of cell division, a concomitant increase of cytoplasmic mass is essential. A massive amount of proteins, such as transcription factors, structural proteins, and ribosomes, has to be synthesized.

Interestingly, defects in factors regulating basic cellular processes frequently lead to distinct growth phenotypes. *Arabidopsis* leaves lacking certain ribosomal proteins commonly exhibit proliferation and ploidy defects leading to a reduced size and a pointed shape (Ito et al. 2000; Horiguchi et al. 2011). In addition, developmental phenotypes typical for defective auxin signaling, like disturbed apicobasal pat-

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terning, have been described in ribosomal-protein mutants (Nishimura et al. 2005; Szakonyi and Byrne 2011). The question how genes with housekeeping functions can influence growth in such a specific way is addressed in a current review by Tsukaya et al. (2013). In the case of ribosomes, not only the total amount of ribosomes but also their composition might be critical for appropriate plant development. As many ribosomal proteins are encoded by small gene families in higher plants, differentially composed ribosomes may have specialized functions, for example, the preferential translation of certain classes of pre-mRNAs or a differential responsiveness to internal or external cues (Barakat et al. 2001; Horiguchi et al. 2012).

Arabidopsis growth is positively correlated with the expression of the evolutionarily conserved kinase target of rapamycin (TOR) (Deprost et al. 2007). In yeast and animals, TOR is known as a nutrition and energy sensor regulating cell growth and lifetime (Fontana et al. 2010). Similarly, the TOR-signaling complex interlinks nutrient state and environmental conditions with translational regulation in plants. *Arabidopsis* mutants with decreased AtTOR activity show smaller cells and organs and plants growing under limited nutrient availability or light energy depletion phenocopy TOR knockdown plants. *Arabidopsis thaliana* TOR (AtTOR) influences the number of translationally active polysomes and binds rRNA gene promoters, thereby stimulating rRNA gene expression (Deprost et al. 2007; Ren et al. 2011). As in animals, AtTOR interacts with Raptor and LST8 homologs to activate the ribosomal protein S6 kinase (S6K), a regulator of cell size that modulates translational capacity by phosphorylation of ribosome components (Meyuhas 2008; John et al. 2011). S6K promotes cell expansion and is required for repression of proliferative growth under nutrient-limiting conditions (Henriques et al. 2010, 2013). A further link between the TOR pathway and cell growth is the TOR-dependent stimulation of factors required for biogenesis and modification of cell walls (Leiber et al. 2010; Caldana et al. 2013). Except for auxin, which has been shown to activate the TOR/S6K-signaling cas-

cade in plants, upstream effectors of AtTOR are largely unknown (Schepetilnikov et al. 2013).

Cell-Autonomous Factors Involved in Cell-Size Control

Few factors are known to regulate cell size cell autonomously. A mechanistic link between cytoplasmic growth and cell proliferation is provided by the TCP class I family member TCP20 and its close paralogs TCP6 and 11. By binding to a specific promoter element, TCP20 activates the expression of many ribosomal genes and cyclin CYCB1;1, which is highly expressed at the G₂/M transition (Li et al. 2005). Depending on the organ and the developmental stage, TCP20 seems to influence cell expansion and cell proliferation differentially. Mutants with altered TCP20 activity show expression changes in many cell-wall-related genes, suggesting an influence on cell expansion by modifying cell-wall characteristics (Hervé et al. 2009). TCP9 was identified as a downstream target of TCP20, but its role in growth processes is currently unknown (Danisman et al. 2012). The dimeric complexes TCP20-TCP8 and TCP20-TCP22 repress the biosynthesis of jasmonic acid (JA), a negative regulator of cell proliferation (Pauwels et al. 2008; Danisman et al. 2012). Underlining the opposing functions of class I and class II TCPs, TCP4 has been shown to positively regulate JA biosynthesis (Schommer et al. 2008). JA inhibits cell proliferation and endoreduplication, but also stimulates many cell growth and cell-cycle-licensing genes (Noir et al. 2013). Thus, increased JA levels could cause the enlargement of leaf cells observed in *tcp20* mutants (Danisman et al. 2012).

Endoreduplication often accompanies plant-cell expansion. *Arabidopsis* leaf cells range from 2C stomate cells to 32C trichome cells, and achieving the correct ploidy level is important to reach proper cell and organ sizes (Melaragno et al. 1993; del Pozo et al. 2006). In addition to its effects on cell proliferation, the class I-type TCP15 represses endoreduplication in *Arabidopsis* cotyledons and trichomes by modulating the expression of cell-cycle genes (Li et al. 2012). Whether endocycling induces ploidy-de-

pendent growth or is merely an accompanying process of the transition from mitotic cell proliferation to the cell expansion and differentiation phase is still a matter of debate (Breuer et al. 2010).

Cell Expansion with a Cell Wall

Cell walls represent the plant's exoskeleton and, together with the central vacuole, enable the generation of large, flat surfaces that are required by organisms depending on photosynthesis (Wolf et al. 2012). Whereas the wall composition might differ between cell types and also between species, the basic architecture is conserved. The stiff but extensible structure of the primary cell wall is provided by neatly arranged cellulose fibrils that are embedded in a matrix of proteins and polysaccharides, such as pectins and xyloglucan. Some tissues form a secondary cell wall including lignin once the final cell size is reached.

Before a plant cell can expand, the rigid exoskeleton has to be loosened. To enhance cell-wall elasticity, modifications, such as pectin demethylesterification are induced by auxin (Peaucelle et al. 2011). Auxin-induced acidification of the apoplast by stimulation of H^+ -ATPases activates expansin proteins (Hager 2003). Expansins support the rearrangement of microfibrils by breaking hydrogen bonds between polysaccharides and are implicated in regulating directional cell expansion in *Arabidopsis* (Cosgrove 2005; Goh et al. 2013). The plant cell continuously controls the integrity of its wall to adjust it to the cellular growth requirements. Therefore, sensors at the plasma membrane are required to monitor the current state. A group of cell-surface receptors is the family of receptor-like kinases (RLKs) in *Arabidopsis* (Shiu and Bleecker 2001; Gouget et al. 2006). RLKs are plasma-membrane-spanning proteins with a cytoplasmic kinase domain and an extracellular ligand-binding domain. Promising candidate receptors for cell-wall integrity signals are the five members of the family of wall-associated kinases (WAKs) because they bind pectins and are required for cell expansion (Kohorn and Kohorn 2012). The ability to bind

oligogalacturonides suggests WAKs are involved in stress-related signaling, as biotic and abiotic stresses often lead to changes of pectin conformations (Brutus et al. 2010). Cell-wall growth and the related signaling events are excellently reviewed by Wolf et al. (2012).

The *Catharanthus roseus* RLK1-like (CrRLK1L) proteins represent a plant-specific family of RLKs involved in cell-wall-integrity signaling and in the control of cell growth (Hématy and Höfte 2008). The CrRLK1L family member THESEUS1 (THE1) limits cell elongation and leaf growth in response to compromised cell-wall integrity in *Arabidopsis* (Hématy et al. 2007). During normal growth, THE1 and its close homologs FERONIA (FER) and HERCULES1 receptor kinase (HERK1) promote brassinosteroid-induced cell expansion (Guo et al. 2009). The activity of THE1 is also required for the excess cell expansion seen in a mutant for the microtubule-depolymerizing kinesin AtKINESIN-13A (Oda and Fukuda 2013). Loss of AtKINESIN-13A function has been proposed to cause uneven cell-wall biosynthesis owing to the documented clustering of Golgi stacks in the mutant (Lu et al. 2005), which would, in turn, activate the THE1-dependent cell-wall-integrity pathway, resulting in increased cell expansion and providing a link between cell-wall-integrity sensing and growth control (Fujikura et al. 2014). Not many intracellular downstream targets of cell-wall regulators have been identified yet. FER has been detected in a complex that activates Rho GTPases (Duan et al. 2010). Thus, it indirectly supports the NADPH-oxidase-dependent production of reactive oxygen species (ROS). Similar cell-wall-related signaling pathways are present in animals and yeast (Wolf et al. 2012).

Heterogeneous growth rates and cell-shape formations cannot only occur at different areas of a leaf epidermis but even at different sides of individual cells, indicating the requirement of constant cell-wall growth coordination and adjustment between neighboring cells (Elsner et al. 2012). To balance growth constraints between growing adjacent cells the microtubule-severing protein katanin is required (Uyttewaal et al. 2012).

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Compensation

In some cell-proliferation mutants, the fascinating phenomenon of compensated cell enlargement can be observed. In these mutants, a reduced cell number can at least partially be offset by increased cell expansion. Conversely, reduced cell expansion can compensate for increased cell numbers in some mutants (reviewed by Horiguchi and Tsukaya 2011). Although the molecular details of compensation remain unclear, in some cases, intercellular communication seems to be involved. Clonal analyses using the *angustifolia3* (*an3*) mutation have shown that cells defective in the putative transcriptional coactivator AN3 can induce compensated cell enlargement even in neighboring wild-type cells (Horiguchi et al. 2005; Kawade et al. 2010). Mutant analyses also provided cell-autonomous scenarios for compensation-related changes in final cell size. In the *Arabidopsis* mutant *fugu2*, the disruption of chromatin assembly factor 1 activates a DNA damage response, leading to decelerated cell cycling and a premature entry into endocycling, which results in the formation of fewer but larger cells (Hisanaga et al. 2013). Conversely, a decrease in cell size can compensate for excessive proliferation, as in the knockout mutant of the endocycle-stimulating Fizzy-related 2 (Larson-Rabin et al. 2009). In the cell-number-defective *oligocellula* (*oli*) mutants, compensation is only induced when different uncompensated *oli* mutations are combined (Fujikura et al. 2009). Because the drop below a certain cell number threshold seems to have triggered the cell-size increase in the multiple *oli* mutant background, an active sensing mechanism determining the cell number has been suggested.

GROWTH REGULATION AT THE ORGAN LEVEL

Ultimately, the growth of individual cells in an organ needs to be coordinated to achieve the correct final size and shape. Although in principle this could be achieved by cell-autonomous mechanisms, in practice it appears more plausible that intercellular signaling is involved in

this coordination, and this view is supported by insights generated over recent years. In addition, the final size and shape of organs depend on their identity, and factors involved in the interplay between organ identity and growth are beginning to be uncovered.

Communication between Cells: Non-Cell-Autonomous Signals

Plant hormones play critical roles in coordinating cellular behavior. In the case of organ growth, auxin stimulates the growth-promoting cascade of ARGOS, ANT, and downstream cell-proliferation factors (Hu et al. 2003). However, how this process contributes to coordinating proliferation throughout organ primordia is currently not well understood. The coordinated arrest of cell proliferation (discussed in Reber and Goehring 2015) has been suggested to involve a mobile growth factor distinct from classical phytohormones that would be produced by the cytochrome P450 *KLUH*/*CYP78A5* at the organ periphery and base (Anastasiou et al. 2007; Kazama et al. 2010). However, as mentioned above, clonal analysis indicates that the range of *KLUH* action is extending beyond individual organs and is, thus, larger than assumed (Eriksson et al. 2010). It appears more likely that the *KLUH*-dependent signal could be used to coordinate the growth of individual organs, for example, within a flower, to help ensure uniformity of the individual organs and, thus, symmetry of the flower as a whole.

Signals derived from the epidermis control the growth of underlying tissues. A brassinosteroid-dependent signal generated in the epidermis has been shown to influence cell expansion in inner leaf layers (Savaldi-Goldstein et al. 2007). Phytosulfokine (PSK), a cell-elongation-promoting peptide signal, can non-cell-autonomously stimulate root and shoot growth when sensed solely in the epidermis, and this effect appears to be mediated via brassinosteroids (Hartmann et al. 2013). Growth-promoting signals can also travel in the opposite direction (i.e., from inner tissue to the epidermis). The aforementioned protein AN3 is expressed in mesophyll cells and moves into the epidermis

to induce cell proliferation (Kawade et al. 2013). Similarly, the receptor-like kinase *ERECTA* and its homologs are expressed in subepidermal tissues in the stem and flower pedicels from where they promote cell proliferation and growth throughout the pedicels (Uchida et al. 2012).

Organ Identity–Dependent Growth Regulators

Most mutants of growth regulators show similar changes in the sizes and shapes of leaves and floral organs, indicating that these homologous organs use the same basic machinery to regulate growth processes. However, the different floral organ types look very different from each other, as they do from vegetative leaves. Therefore, organ identity appears to modulate the activity of the basic growth-control machinery to modify size and shape according to the organ's functions. Several factors linking organ identity and growth patterns have been identified by now.

A petal-specific isoform of the transcription factor *BIGPETAL* (BPEP) limits petal cell size in interaction with *AUXIN RESPONSE FACTOR8* (Szécsi et al. 2006; Varaud et al. 2011). In contrast, the transmembrane kinase (TMK) subfamily of RLKs promotes cell expansion and proliferation in leaves but not in petals (Dai et al. 2013). Thus, TMK defects cause a reduced leaf size while the flower size is unimpaired. Opposite organ-size defects have been shown in the poly(A) polymerase mutant *paps1*, which produces small leaves attributable to a cell-size defect and larger petals attributable to an increased number of cells, resulting at least partly from an increase in the number of founder cells in the petal anlage (Vi et al. 2013; Trost et al. 2014). At the molecular level, *PAPS1* has been suggested to polyadenylate specific subsets of pre-mRNAs that vary between the different organs.

Petals and leaves grow along different polarity fields that are defined by PIN1-dependent polar auxin transport. The proliferation-stimulating transcription factor *JAGGED* (*JAG*) modulates the shape-determining auxin distribution depending on the identity of the organ (Sauret-Güeto et al. 2013). *JAG* promotes anisotropic growth of petal cells and *jag* mutants

show serrated leaves and narrow, small petals (Dinneny et al. 2004; Schiessl et al. 2012).

Growing to a Distinct Leaf Shape

The vast diversity of leaves and flowers in nature is not only characterized by differences in size but also by many different shapes. The shape of a mature leaf closely correlates with a variation in cell number (Poethig and Sussex 1985). Several mutants with altered length to width ratios show that anisotropic cell expansion contributes to shape formation as well. Leaf-width regulators in *Arabidopsis* are *ANGUSTIFOLIA* (*AN*) and *AN3*, while two redundant *LONGIFOLIA* genes and the cytochrome P450 *ROTUNDIFOLIA3* influence leaf length (Tsuge et al. 1996; Horiguchi et al. 2005; Lee et al. 2006). Most of these factors act independently in different pathways.

The degree of dissection of the leaf margin varies widely between different species, ranging from smooth to serrated and increasingly deeply lobed (Efroni et al. 2010; Nicotra et al. 2011; Byrne 2012). Dissection requires the definition of sites where the leaf margin grows out (i.e., the tips of serrations and lobes) and the inhibition of growth in the intervening sinuses. The sites of outgrowth are defined by auxin maxima, which are established by polar auxin transport and reinforced by suppressing a mechanism that allows reorientation of auxin transport around the maxima (Bilborough et al. 2011). Growth in the sinuses between serrations or lobes is suppressed by NAC-family and homeodomain transcription factors (Kawamura et al. 2010; Sicard et al. 2014; Vlad et al. 2014). Strikingly, despite its prominence in nature, the function of variation in leaf-margin dissection remains largely unknown (Nicotra et al. 2011).

SUMMARY AND OUTLOOK

Over the last two decades, our understanding of growth and size control has been broadened continuously. Next to diverse factors working in independent pathways, several key regulators have been identified in recent years. In addition, the fundamental and diverse roles of phytohor-

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mones, such as auxin and gibberellin, but also JA, brassinosteroids, and ethylene are being unraveled in more and more detail (Nelissen et al. 2012; Noir et al. 2013; Pei et al. 2013; Sauret-Güeto et al. 2013; Zhiponova et al. 2013). Open questions in organ-size regulation continue to be addressed by diverse approaches. Next to mutant characterization, the continuous advancement of imaging techniques enables more detailed insights into cellular growth processes (Schiessl et al. 2012). In addition, gene expression studies and modeling approaches provide powerful tools to analyze growth dynamics (Andriankaja et al. 2012; Sauret-Güeto et al. 2013). Also quantitative trait locus (QTL) analyses, which use the natural variation of shapes and sizes between often closely related species, might provide new insights into growth regulation and evolutionary aspects of size control (Kelly and Mojica 2011; Sicard et al. 2011).

To our current knowledge, many growth-regulating factors and pathways seem to act independently, reflecting the intricate genetic networks used by plants to adjust their growth to diverse environmental conditions. Somehow, the information derived from all these pathways is integrated to ensure that leaves and petals grow to their optimal shapes and sizes that we are so familiar with. Understanding how this integration is achieved will be one of the major challenges for the field in the future.

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