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Control of Organ Size in Plants

Review

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The size of plant organs, such as leaves and flowers, is determined by an interaction of genotype and environmental influences. Organ growth occurs through the two successive processes of cell proliferation followed by cell expansion. A number of genes influencing either or both of these processes and thus contributing to the control of final organ size have been identified in the last decade. Although the overall picture of the genetic regulation of organ size remains fragmentary, two transcription factor/microRNA-based genetic pathways are emerging in the control of cell proliferation. However, despite this progress, fundamental questions remain unanswered, such as the problem of how the size of a growing organ could be monitored to determine the appropriate time for terminating growth. While genetic analysis will undoubtedly continue to advance our knowledge about size control in plants, a deeper understanding of this and other basic questions will require including advanced live-imaging and mathematical modeling, as impressively demonstrated by some recent examples. This should ultimately allow the comparison of the mechanisms underlying size control in plants and in animals to extract common principles and lineage-specific solutions.

On the Importance of Size and Form to a Plant

A typical seed plant can be described as a collection of repeated parts [1]. There may be one or more stems from which a series of leaves and flowers radiate, the flowers themselves symmetrical arrangements of sepals, petals, and reproductive organs. The size reached by these various component parts as well as the plant as a whole represents a complex integration of environmental and genetic influences [2]. Plants must calibrate growth to environmental conditions to make strategic use of limited resources and to compete with neighbors. This could mean, for example, accelerating skyward growth in order to avoid being shaded by a neighbor. Although the environment strongly influences organ growth and final size, it can do so only within genetically specified limits, i.e. even under optimal conditions daisies will never grow as large as sunflowers. The correct regulation of growth ensures that organs essential to survival and reproduction function properly. For example, plants that rely on animal pollinators form attractive floral structures, often including large petals and/or complex three-dimensional shapes [3]. Also, the various components of the flower must achieve a coordinated geometry to ensure efficient pollen transfer and receipt [4].

Modes and Phenomena of Growth in a Plant

Plants have the potential for indeterminate growth throughout their lifespan. For example, the trunk of a tree continues to widen, or plants that spread asexually through runners can grow to cover very large areas. In particular, roots and stems exhibit indeterminate growth, whereas determinate organs such as leaves, sepals, and petals adopt a more defined final form and size [1]. Here, we limit ourselves to a discussion of size control in the determinate organs, referring mainly to leaves and flowers of the dicotyledonous model plants *Arabidopsis thaliana* and *Antirrhinum majus* (snapdragon).

The organs of a plant develop from reservoirs of pluripotent cells that are maintained throughout the life of the plant in specialized tissues called meristems. Of these, the shoot apical meristem gives rise to all aerial tissues of the plant [5]. Organ initiation is triggered by a local maximum of signaling of the phytohormone auxin [6]. At the site of organ initiation, cell walls become more elastic due to pectin demethylesterification and possibly other cell wall modifications [7], allowing the outgrowth of a bulge that becomes the organ primordium (Figure 1).

The organ primordium initially consists entirely of cells undergoing coordinated division and expansion. During this period, which we will refer to as growth by proliferation, cell size remains largely constant and the cells stay densely cytoplasmic, indicating the doubling of cytoplasmic mass between divisions. Expansion of plant cells necessarily requires a loosening of the cell wall and concomitant water uptake to a vacuole of high osmolarity to maintain turgor. At the same time the expanded cell wall requires apposition of new cell-wall materials to counteract the loosening that occurred [8,9]. As the organ grows, distal cells cease cell division and enter a phase of postmitotic expansion, where the cells enlarge and become highly vacuolated compared to the cells in the meristem or the proliferating region of the primordium (Figure 1). This phase of growth by exclusive cell expansion (which we will refer to simply as expansion) is often accompanied by ploidy increase through endoreduplication [10]. The cell wall may also undergo further remodeling, including the production and trafficking of polysaccharides and cell wall-associated proteins [8]. The transition from proliferation to expansion has often been described as an 'arrest front' moving from the tip towards the base of the leaf (e.g. [11]). However, more recent studies suggest that the proliferative region anchored at the base of the leaf remains a relatively constant size for some days before rapidly disappearing. Under this model the growth occurring at the base of the leaf displaces older cells distally until they fall outside the zone of proliferative competence and thus cease to divide [12,13]. In particular, cells in a narrow region at the junction between the blade and the petiole have been proposed to supply proliferating cells in a bidirectional manner for these two parts of the leaf [14].

Thus, two main processes, cell proliferation and cell expansion, underlie final organ size. In theory, alterations in organ size can result from a change in the duration of either of these growth phases, in the rates of cell proliferation or cell expansion, in the size of the proliferative zone within the growing organ, or in the number of cells initiating the primordium. Regarding the first case, many mutants that form larger or smaller organs show changes in the timing of proliferation arrest, resulting in more or fewer cells that expand to their 'normal' size (see below), suggesting that the timing of this transition is a critical step in size control (e.g. [15–17]). Regarding regulation of cell proliferation or expansion rates, limited examples support this mechanism of growth control

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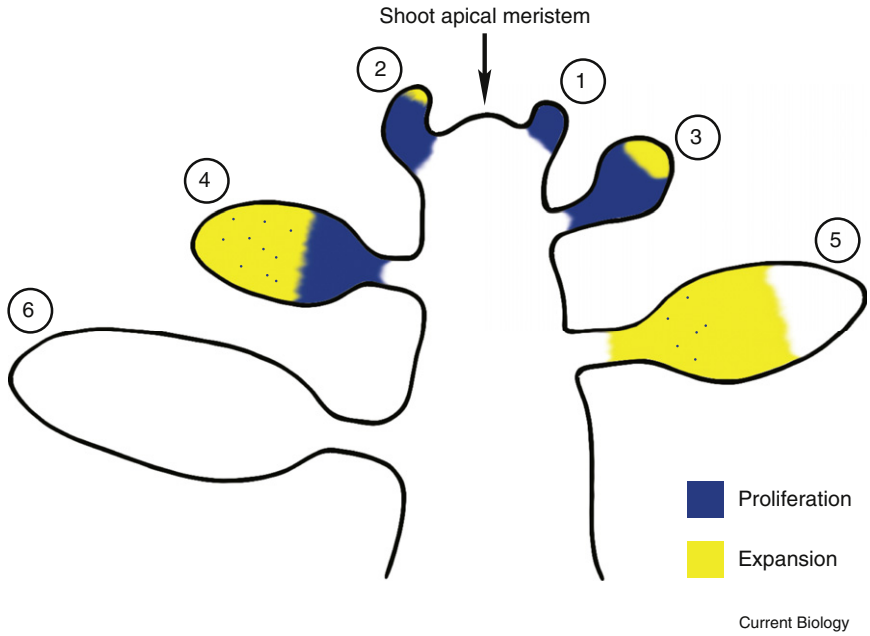

Figure 1. Schematic representation of leaf growth in plants.

Leaves are initiated as primordia at the shoot apical meristem (SAM). During the earliest stages of leaf growth (stage 1), all of the cells in the primordium proliferate (indicated by blue color). Proliferation ceases first at the very tip of the primordium (stage 2), giving way to cell expansion (yellow). The region of cell proliferation near the base of the leaf stays relatively constant for some time (stages 3 and 4), while more distal cells that have grown out of this proliferative zone undergo cell expansion; exceptions are the dispersed meristematic cells (stomata and vascular precursors) that continue proliferation for a longer period of time (blue dots in stage 5). The basal proliferative zone then disappears (stage 5), and the leaf continues to grow by cell expansion until the final size has been reached (stage 6).

as well (e.g. [18,19]). For the third case, recently one example has been described in which a repressor of leaf growth restricts the size of the proliferative zone and in this way controls final organ size [20]. Examples for the fourth case are known in maize [21], yet to our knowledge it has not been clearly demonstrated in a dicotyledonous species that the number of cells initiating organ primordia is regulated in the control of organ size.

Monitoring and Measuring the Size of the Growing Organ

A central problem in growth control that remains essentially unsolved in plants is how proliferation is terminated at the appropriate time to ensure the target cell number and, ultimately, organ size are reached. Given the above description of leaf growth, there are two aspects to this question. First, how is the size of the proliferating region at the leaf base determined? And second, how is proliferation in this region arrested once enough cells have been produced? The size of the proliferating region could be determined, for example, by a gradient of a mobile signal produced at the blade/petiole junction that could determine the maximum distance from the junction at which cells still proliferate; proliferation would only occur near the junction where the signal concentration is above a threshold [13].

The timing of proliferation arrest could, in principle, be determined in a cell-autonomous or a non-cell autonomous manner. Cell number in an organ could be counted via the level of a molecule that accumulates or is degraded according to cell divisions [22], perhaps primarily in the cells at the blade/petiole junction. Once the level of this molecule rises above or falls below a certain threshold, proliferation would arrest. Alternatively, the size of the primordium may be measured more directly, for example again using gradients of extracellular signals or the dilution of a mobile growth factor. Unfortunately, despite the identification of several genes that affect final organ size through an influence on cell proliferation, our understanding about the above issues is still in its infancy. The same applies to our understanding of how cell expansion is stopped at an appropriate cell size.

Genes and Genetic Pathways Controlling Size

The control of size, and by extension form, is a principle way in which a plant can adapt to environmental conditions. Thus, size is expected to be a complex trait, similar to most yield-related traits [23,24]. Molecular evidence to date supports this view. While many genes have been identified as playing a role in organ size control, relatively few coalesce into pathways (Figure 2). So far, mainly two pathways have emerged and these both act to maintain cell proliferation in the primordium. It should be kept in mind, however, that although genes are generally described as acting on proliferation or expansion based on the cellular changes observed in mutant organs, in many cases the precise mode of action and the primary targets are still unclear.

Candidate Cell-Autonomous Factors

In this section we will first hightlight some genes inhibiting the transition from cell proliferation to cell expansion and then present some which promote this transition (summarized in Figure 2). As noted above, auxin signaling is required for organ initiation. Several cell-autonomously acting effectors of the auxin response in organ biogenesis have been identified. One such factor is the protein encoded by *EBP1* (*ErbB-3 epidermal growth factor binding protein*), which is stabilized by auxin. Human *EBP1* is required for ribosome biogenesis, and a similar role could be expected in plants. Manipulation of plant *EBP1* activity has demonstrated a role in promoting organ growth by acting both on the rate and duration of cell proliferation. *EBP1* stimulates the expression of *CyclinD3;1* (see below) [25].

In growing organs, auxin stimulates the expression of *ARGOS* (*AUXIN-REGULATED GENE INVOLVED IN ORGAN SIZE*), a gene encoding an ER-localized protein of unknown function [26,27]. Overexpression of *ARGOS* results in larger aerial organs, whereas reduced *ARGOS* expression leads to smaller organs. The differences in organ size are mainly due to changes in cell number caused by alterations in the duration of the cell proliferation period. *ARGOS* promotes expression of another size-regulatory gene, *AINTEGUMENTA* (*ANT*), encoding a member of the AP2/ERF transcription factor

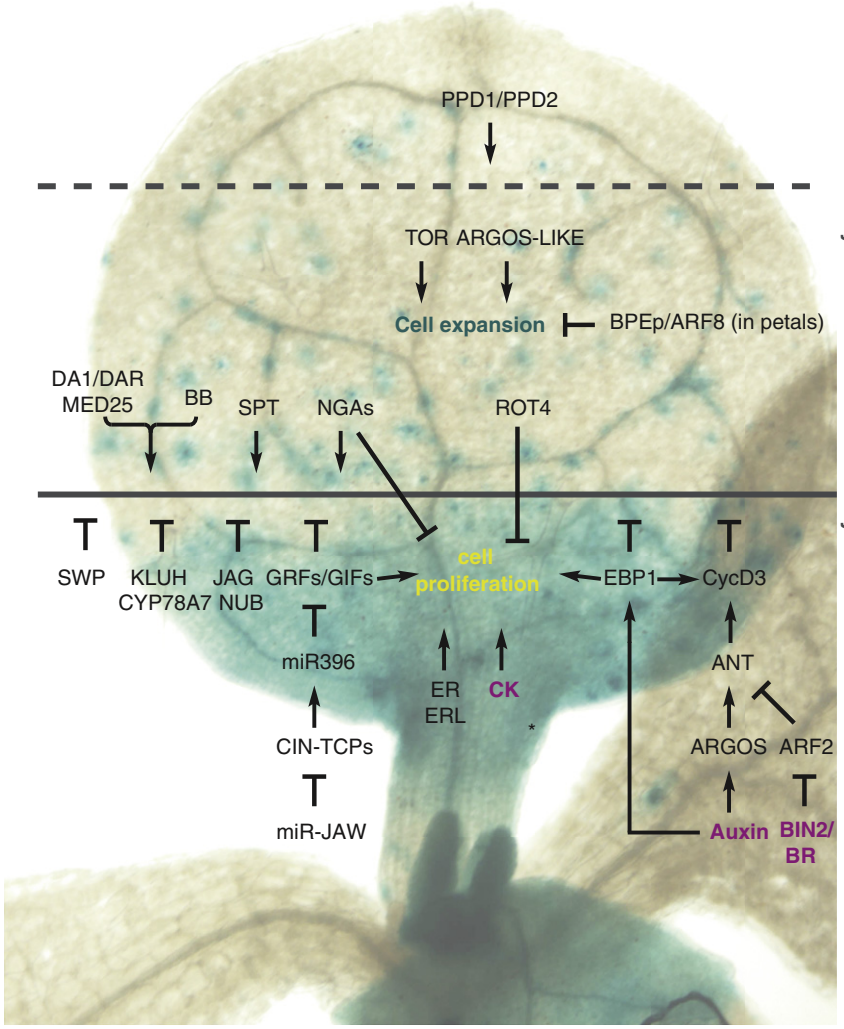

Figure 2. Summary of genetic factors and pathways influencing lateral organ size in plants.

A GUS-stained leaf of a transgenic *A. thaliana* line expressing the mitotic marker pCycB1;1::CDBGUS is shown; blue staining indicates mitotically dividing cells. Approximate positions of the primary, general proliferation arrest front and of the second arrest front that relates to dispersed meristematic cells are shown. Factors shown above the arrest-front lines with arrows pointing towards them promote proliferation arrest, while factors below the primary arrest-front line with T-bars towards the arrest front act to maintain cells in a proliferative state. Arrows or T-bars pointing directly towards 'cell proliferation' indicate an influence on the rate of proliferation, rather than on its timing. Arrows or T-bars pointing towards 'cell expansion' indicate a promoting or repressing influence, respectively. Asterisk indicates the position of the blade/petiole junction. The evidence for a role of the factors not discussed in detail in the main text is from the following references: ER/ERL, ERECTA/ERECTA-LIKE [85]; JAG/NUB, JAGGED/NUBBIN [86–88]; NGA, NGATHA [89]; ROT4, ROTUNDIFOLIA4 [18]; SWP, STRUWWELPETER [90].

family. Changes in *ANT* activity lead to analogous effects as seen for *ARGOS* misexpression, and *ANT* in turn maintains the expression of the D-type cyclin *CYCD3;1*, whose loss together with that of two closely related D-type cyclins causes premature termination of cell division in leaves (even though overall leaf size is unchanged in *cycd* triple mutants) [27–30]. Thus, these proteins work together in a signaling pathway to sustain the cell proliferation phase of growth.

The *ANT* pathway also intersects with another arm of the auxin response. A family of transcriptional regulators, the *AUXIN RESPONSE FACTOR*s, mediate the transcriptional response to auxin. Of these, the transcriptional repressor *AUXIN RESPONSE FACTOR2* (*ARF2*) limits cell proliferation and thus organ size by repressing the activity of *ANT* and *CYCD3;1* [31–33]. ARF2 protein can be inactivated by phosphorylation by the brassinosteroid-activated BIN2 kinase. This hints at a cross-talk between auxin and brassinosteroids in growth control [34].

A second pathway promoting cell proliferation involves transcription factors of the TCP (TEOSINTE BRANCHED1, CYCLOIDEA, PCFs) and GROWTH-REGULATING FACTOR (GRF) classes, two redundant multi-gene families in *Arabidopsis*. The importance of the TCP family in growth control became clear first from the *cincinnata* (*cin*) mutant in snapdragon (*Antirrhinum*) and the *jaw-D* activation-tagged mutant of *Arabidopsis* [35,36]. In both these mutants, leaves overgrow to a highly crinkly shape because of excess cell proliferation particularly at the leaf margins. *CIN* encodes a member of the TCP family. In *cin* mutants, the size of the proliferative region at the leaf base seems to be enlarged and its distal boundary is concave, such that cells at the leaf margin still proliferate at positions where cells in the center have already arrested proliferation. In the *jaw-D* mutant, overexpression of the microRNA *miR319a* downregulates five genes of the *TCP* family. Downregulation of another three family members causes even more severe phenotypes of overproliferation in leaves, while miR319-resistant versions of TCPs and loss-of-function mutations in *miR319a* reduce organ size and cause premature cell differentiation [37,38]. In fact, promoting cell differentiation has been proposed as the primary function of TCPs, rather than directly arresting proliferation [39]. TCP4 in turn promotes the expression of another microRNA, miR396, which targets seven of the nine members of the *GRF* gene family. GRFs, together with the family of putative transcriptional co-activators encoded by the *GRF-INTERACTING FACTOR* (*GIF*) genes regulate organ size by maintaining cell proliferation, with mutants forming smaller and narrower leaves, whereas overexpression leads to larger leaf size [19,40–43].

In addition to these emerging pathways, a considerable number of additional genes have been identified that contribute to the control of final organ size by promoting the transition from proliferation to expansion. Both the putative ubiquitin-binding protein DA1 and the E3 ubiquitin-ligase

BIG BROTHER (BB) limit organ size by promoting the exit from proliferation. The synergistic phenotype of the *da1 bb* double mutant suggests that their activities may converge to target the same growth-stimulating factors for proteasomal degradation [16,17]. Also the mediator subunit Med25 has been implicated by genetic analysis to act redundantly with DA1 [44].

While the previous sections referred to bulk proliferation of epidermal and mesophyll cells, distinct types of cells, such as stomatal and vascular precursors, continue to divide in more distal regions of the leaf where general proliferation has already terminated. These cells are known as dispersed meristematic cells (DMCs), and their proliferation arrest is under control of the putative transcription factors PEAPOD1 (PPD1) and PPD2, with *ppd1 ppd2* double mutants forming larger, bell-shaped leaves due to increased lamina growth relative to the leaf margin [11]. This has led to the suggestion that subsequent to the primary arrest front there is a secondary arrest front that terminates DMC proliferation.

In contrast to the increasing list of factors influencing cell proliferation, we know much less about the regulation of growth by cell expansion. The *ARGOS* homologue *ARGOS-LIKE* (*ARL*) promotes organ growth via cell expansion downstream of brassinosteroid-signalling [45]. Similarly, the kinase encoded by the *TARGET OF RAPAMYCIN* (*TOR*) gene is required for cell expansion in leaves, and overexpression increases leaf size due to the cells becoming larger [46,47]. As in yeast and animal cells, plant *TOR* may link cell growth with the availability of metabolic resources [48]. In petals, cell expansion and petal size are limited by the activities of the bHLH transcription factor BIGPETALp (BPEp) and ARF8 [49,50].

Candidate Non-Cell Autonomous Factors

Based on their molecular nature as transcriptional regulators or intracellular enzymes, all of the above proteins are likely to function in a largely cell-autonomous manner. However, the requirement that growth be coordinated across small and large distances, between tissue layers, organs, and sectors thereof strongly suggests that non-cell autonomous factors are necessary to facilitate this coordination. The dramatic phenotypes of TCP loss-of-function mutants illustrates, for example, how leaf margin and leaf center growth must be tightly integrated to preserve the flat shape of the leaf. Similarly, the crinkled leaves of variegated horticultural varieties that consist of both wild-type green and mutant white tissue result from differential growth of the two tissue types (Figure 3).

Experimental evidence supports the existence of non-cell autonomous factors acting to control size. One such piece of evidence is the phenomenon of 'compensation' [51–53]. Compensation is seen in some mutants where the primary defect is in cell proliferation or expansion, but where the other process can be adjusted in a compensatory manner to minimize changes to the final size of the organ. In particular, it appears that cell number needs to fall below a critical threshold for compensation to be triggered [54], and sector analysis suggests that this trigger involves a non-cell autonomous signal [52]. The same basic phenomenon has been observed in *Drosophila* wings, where experimentally induced changes in cell division and cell number were offset by opposite changes in the size of the individual cells, leading to wings of normal shape and size [55]. These observations together suggest that, in both plant and animal systems, organ size can be regulated at least partly independently of cell number.

Figure 3. Leaf shapes resulting from differential growth in chimaeras.
Variegated leaves from horticultural varieties of *Hosta* sp. (left) and *Ficus* sp. (right) are crinkled and distorted because of more growth of the green, photosynthetically active tissue relative to the mutant white tissue.

A plant-specific aspect of organ growth that suggests the involvement of non-cell autonomous signals derives from the composition of plant organs of clonally distinct cell layers (i.e., the epidermis and one or more subepidermal layers), whose growth needs to be coordinated [1]. Related to this is the question whether the different cell layers are all equally important for determining organ size and shape. Periclinal chimaeras of tobacco where the genotype of the epidermis is different from that of the subepidermal tissue indicate that differences in cell proliferation in the epidermis can modify the extent of proliferation of subepidermal cells [56]. Similarly, transgenic lines in which only the epidermis can perceive brassinosteroids or is depleted of active brassinosteroids demonstrate a non-cell autonomous role of the epidermis in modulating cell expansion in the subepidermal tissue [57]. Together, this supports an important role for the epidermis in controlling leaf size and shape, even though other experiments also suggest some degree of autonomy of the subepidermal cells regarding the arrest of cell division [58].

Phytohormones as mobile signals are obvious candidates for coordinating growth and possibly monitoring organ size [59], although it is currently not known in detail whether and how they are related to any of the tissue-wide phenomena outlined above. The influences of auxin and brassinosteroids on organ growth have been described above. In addition, cytokinins promote organ growth by stimulating cell proliferation, with cytokinin depletion or overproduction resulting in smaller or larger leaves and flowers, respectively [60,61]. Similarly, gibberellins promote growth via expansion and/or proliferation, acting to repress the activity of the growth-restraining DELLA proteins [62,63]; DELLA factors may be particularly important in adjusting growth in response to environmental influences [64]. In addition to these classical phytohormones, a novel mobile signal whose synthesis depends on the related cytochrome P450 enzymes KLUH/CYP78A5 and CYP78A7 promotes leaf and floral-organ growth. Analysis of chimaeric plants indicates that the presumed signal is integrated

across flowers, suggesting that it may be used to coordinate growth within and between individual organs [15,65–67]. Several other factors have been shown to contribute to size control (Figure 2), yet space constraints preclude their more detailed discussion here.

Approaches to the Study of Size Control in Plants

Most of the information we have to date about the genetics underlying organ size control comes broadly speaking from a reductionist approach. Mutagenesis screens have been very successful, although not without complications. For example, many genes can affect the final size of the plant by being effectors of size control, i.e. by being required for basic cellular processes underlying growth, but not directly controlling size themselves. To get around this difficulty some studies have focused on mutations that affect only certain organs, such as leaves or petals; on mutations that cause an increase, rather than a decrease in organ size; or on mutations that affect size only in one axis (e.g., proximo-distal) under the presumption that such effects are more likely to be specific (e.g. [16,18]). As with every other mutagenesis screen, the problem that genetic redundancy often obscures the effects of individual genes also hampers screens for size-regulatory factors (e.g. [17]).

As a related approach, analyzing the vast natural variation in organ size using quantitative trait locus (QTL) mapping or genome-wide association studies holds great promise for identifying important regulators of size. Until recently this was challenging due to the limited genomic data available for most plant species, yet these limitations are being overcome by the use of next-generation sequencing. To date this approach has identified a small number of genes affecting size. For example, the *fw2.2* gene from tomato affects fruit size by modulating cell proliferation [68], while the *se2.1* gene influences the length of the style by acting on cell expansion [69]. The maize counterparts to *fw2.2* also seem to control size through cell number [70]. Similarly, QTL mapping within or between closely related species that differ in key traits might yield information about targeted functional changes to size control genes (e.g. [71,72]). As changes to organ size in natural evolution are often specific to either leaves or flowers, in contrast to the mostly general effects caused by induced mutations, understanding the genetic basis of such size changes will likely provide insight into the link between organ identity and growth control, a largely unsolved problem in plants and animals. By performing this type of study in parallel and in multiple species pairs, the various solutions adopted in different taxa could illuminate either general principles or unfounded assumptions about how size control works.

The expansive data sets made possible by modern biotechnology and systems biology should at first glance vastly accelerate our understanding of processes such as size control. For example, transcriptome analyses of leaves undergoing the transition from proliferation to expansion have provided a comprehensive list of genes whose expression changes during this critical time window for controlling final leaf size [73]. However, our limited knowledge of the function of much of plant genomes remains a fundamental hindrance. Also, the sheer amount of data in combination with the limited knowledge about gene functions often makes it difficult to identify the important regulatory components for in-depth functional analysis.

Another approach that can lead to a deeper understanding of size control is mathematical modeling in combination with experimentation (often involving advanced imaging techniques) [74,75]. Organ growth is not only a question of gene action but also a physical process, where mechanical forces can act as system feedback. Spatially varying gene expression patterns or a non-homogeneous distribution of mobile growth factors can induce differences in growth rates and/or orientations. Due to the connectedness of plant cells, these differences will lead to deformations of the tissue and potentially the accumulation of stresses that can in turn feed back on gene expression, cellular behaviour or the distribution of signals [76,77]. The results of such dynamic interactions are very difficult to predict or understand by intuition alone. Therefore, mathematical modelling is emerging as a central approach for describing growth and the formation of shape in plant organs.

The modelling approach has been fruitfully applied to several growth-related problems in plants, although not yet to the issue of how growing organs could monitor their size. Several instructive recent examples illustrate the general approach, the critical prerequisites, and the value of modelling to summarize a body of experimental results and to predict non-intuitive aspects of a process that can then be tested experimentally. One study addressed the establishment of the distinctive pattern of cell size in the epidermis of *Arabidopsis* sepals where giant cells are interspersed between smaller cells [78]. Based on live imaging followed by modelling, it was concluded that the cell-size pattern results from the cell-to-cell variability in the timing of division arrest. Skewing this timing by modulating the activity of cell-cycle regulators resulted in the predicted changes to the pattern. Another example studied the development of the complex form of snapdragon flowers [79]. In this case, the model was constrained by data from 3D-imaging of the flower buds and by growth parameters that were extracted from clonal analysis, and the activities and expression patterns of known shape genes were incorporated. However, the shapes of wild-type and different mutant flowers could only be reproduced when individual genes simultaneously influenced both growth rates and growth orientations, but not when they acted on growth rates alone. This finding led to the suggestion that combinatorial effects of spatially varying growth rates and tissue polarities underlie many of the complex three-dimensional shapes in plant organs [77]. As these examples illustrate, modelling of plant morphogenesis relies on detailed, time-resolved information about parameters of cellular and tissue growth (growth rate, orientation, frequency and distribution of cell divisions, etc.) and ideally knowledge about critical genes involved in the process and their interactions; the various mathematical strategies for modelling have been reviewed elsewhere [74]. As underlined by examples from the animal field (e.g. [80]), such approaches can be very fruitful for understanding size control and their application to the problem of plant organ-size control is eagerly awaited.

Ultimately, mathematical models will allow the control of size to be considered as an engineering problem from a theoretical standpoint (reviewed in [81]). This kind of analysis aims to understand how a biological system meets certain performance goals. For example, one would like to know how precisely repeated iterations of the same developmental program achieve the same outcome, such as a constant organ size. Also, it is of interest how robustly the system can reproduce the desired result in the face of stochastic fluctuations in signal concentrations, random

variation in environmental factors or genetic mutations. Several examples, mainly from problems of patterning in animals, indicate the utility of this approach for a deeper understanding of the design and function of biological systems, and applications to questions of size have also produced very encouraging results [82,83].

Outlook

Many open and difficult questions remain when it comes to understanding the control of size in plants. Size is a fundamental property of cells, organs, and body plans. How the complex calculation and execution of achieving the correct size is carried out biologically is both an intellectually compelling problem and one of critical importance to crop science. Up until now much about size control that has been elucidated in other systems, like yeast or animals, has not applied in a direct way to the plant system, outside of genes involved in basic cellular processes like cell cycle control or ribosome biogenesis. Due to the deep evolutionary divergence, however, the signaling pathways involved appear to be largely distinct. Beyond this evolutionary distance, though, there are many obvious differences between plant and animal development and form. A primary difference is the indeterminate and mostly asymmetrical body plan of plants compared to the bilaterally symmetric and determinate form of most animals in at least one stage of their life. This fundamental difference in form could be expected to lead to different solutions in engineering size control. For example, one could hypothesize that plants could tolerate a lower level of precision and robustness in developmental programs, because individual organs of the plant are often not essential to the whole. This principle is well-illustrated by the plant response to infection, which is suicide of the infected area and surrounding healthy tissue, which can lead to whole organ loss [84]. Such a response would require massive regeneration in a bilaterian, but plants can survive loss or partial loss of organs due to their iterative growth pattern. On the other hand, due to their sessile nature, plants must be exquisitely sensitive to environmental signals. Therefore, one might expect that the pathways integrating environmental information into growth control to be highly elaborated and robust. Whether these hypotheses are correct requires a much better understanding of the control of size. However, the point stands that the comparisons would be very interesting to evaluate the strengths and weaknesses of the solutions found in different systems.

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