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# Cell Size Control in Plants

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## Keywords

cell size homeostasis, cell cycle, polyploidy, genome size, meristems, plant growth

## Abstract

The genetic control of the characteristic cell sizes of different species and tissues is a long-standing enigma. Plants are convenient for studying this question in a multicellular context, as their cells do not move and are easily tracked and measured from organ initiation in the meristems to subsequent morphogenesis and differentiation. In this article, we discuss cell size control in plants compared with other organisms. As seen from yeast cells to mammalian cells, size homeostasis is maintained cell autonomously in the shoot meristem. In developing organs, vacuolization contributes to cell size heterogeneity and may resolve conflicts between growth control at the cellular and organ levels. Molecular mechanisms for cell size control have implications for how cell size responds to changes in ploidy, which are particularly important in plant development and evolution. We also discuss comparatively the functional consequences of cell size and their potential repercussions at higher scales, including genome evolution.

## 1. INTRODUCTION

**Ploidy:** number of genome copies within a single cell

**Homeostasis:** tendency to maintain a state of equilibrium, typically through feedback loops that counteract external perturbations

**Autophagy:** regulated disassembly of unnecessary or defective components of the cell for recycling or to produce energy during starvation

Size is important for cell function and is characteristic of different species and of different cell types in the same organism (78, 104). How cell size is specified has intrigued researchers ever since cells became visible to biologists. Early experiments using sea urchins of different ploidy revealed that cell size is strongly affected by nuclear contents (9), while experiments using protozoans showed that progression through cell division depends on cytoplasmic volume (47). The control of cell size through coordination between cell growth and division has been studied genetically (83) and modeled mathematically (34) since the 1970s. This early work, focused mostly on unicellular organisms, established the current intellectual framework and approaches, as detailed in Section 2 (3, 29, 117). Even with this long history, the molecular mechanisms that set and maintain eukaryotic cell size are a major aspect of biology that remains controversial (72, 78, 104). A related, long-standing question is why cell size correlates with genome size and ploidy.

In multicellular organisms, cell size regulation is particularly complex, partly because cell-autonomous mechanisms for size regulation (55, 71, 107, 123) overlap with developmental control of growth and cell division by intercellular signals (20, 72). In spite of this additional complexity, there are important questions related to cell size control that can be addressed only in a multicellular context, in particular the consequences for organ growth, patterning, and differentiation. Plants are a good multicellular system for studying the regulation of cell size. As plant cells cannot migrate because they are encased in a cell wall matrix, their growth and division can be readily tracked and measured by live cell imaging and three-dimensional (3D) image analysis (55, 107, 123). Plants are also particularly relevant for studying how cell size relates to ploidy and to organ size because of the prominent role of polyploidy in plant evolution (97), plant development (112), and crop domestication (99).

Here, we discuss plant cell size control in the context of what we know for other organisms. We first provide an overview of our understanding of cell size homeostasis across eukaryotes and then discuss specific features in plant cells before focusing on how cell size control changes during development and on the functional relevance of cell size and its evolutionary significance. In most of the work we discuss, cell size is defined as a geometric measurement (cell volume, cell area, or length in the case of simple rodlike cells such as fission yeast) and, in some cases, as a correlated biochemical quantity (protein content, protein synthesis rate). For simplicity, we refer to all of these as cell size, except when the particular feature considered as size affects the interpretation of the experiments or models.

## 2. EVIDENCE FOR CELL-AUTONOMOUS SIZE CONTROL IN EUKARYOTES

The characteristic and heritable cell sizes in different species and in different tissue types obviously reflect their genetic makeup; however, this does not necessarily imply that cell size is directly sensed and adjusted. In principle, predictable cell sizes could be achieved if cell growth and processes that reduce cell size, such as division and autophagy, were regulated in parallel in response to external cues, such as nutrient or hormone levels (72).

There are, however, good reasons why cells need to regulate their own size. One reason is endogenous variability, for example, resulting from unequal cell divisions (107, 113) and fluctuation in the number of molecules of key cell cycle regulators (25). This variability would be amplified over generations if growth rate were proportional to cell size (i.e., exponential) and cells divided at intervals independent of their size (2, 80, 115). The buildup of variability over cell divisions can be prevented by linking cell cycle progression to cell growth; this link has been well documented in budding and fission yeast (28, 33, 54, 80, 81, 83, 115, 124, 125). In contrast, if cells grew by a

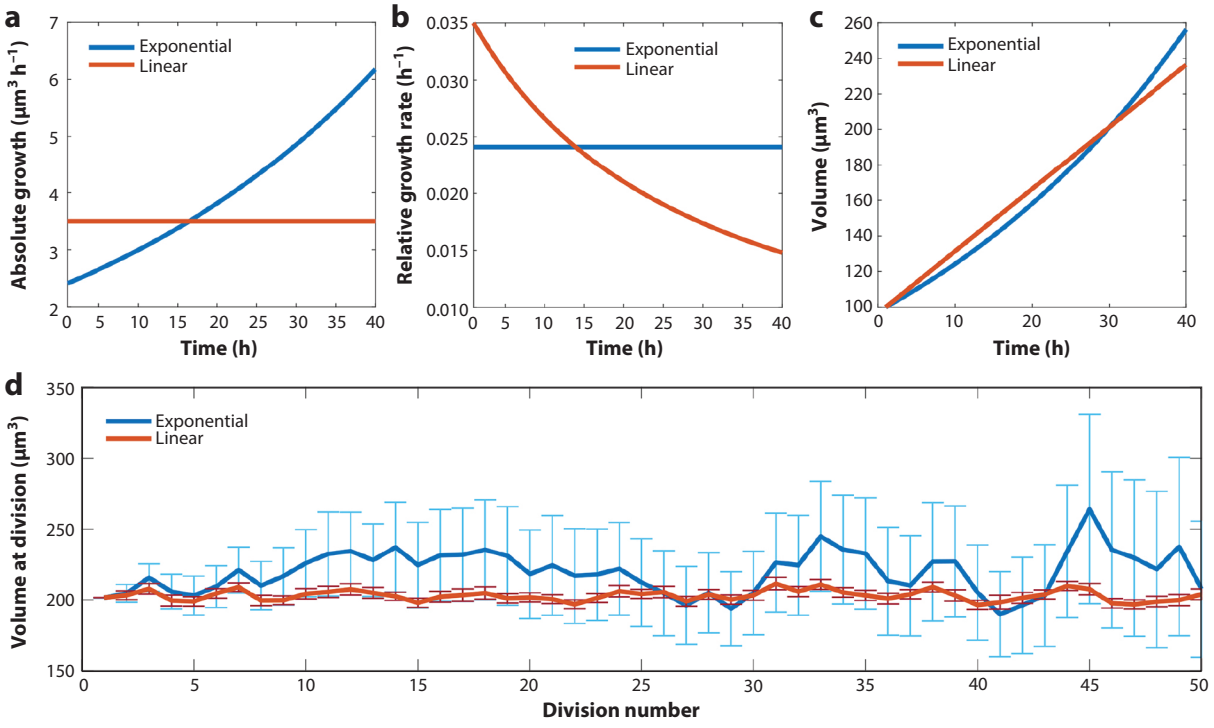


**Figure 1**

Exponential and linear cell growth and their consequences for variability in cell size, shown by computer simulations. (*a*) The absolute increase in cell volume per unit of time is constant for linear growth but increases during exponential growth. (*b*) Growth rate relative to cell volume is constant for exponential growth but diminishes over time if cells grow linearly. (*c*) Exponential and linear growth can result in relatively small differences in cell volume over time, making them difficult to distinguish experimentally. (*d*) Size variability in simulated cell populations growing linearly or exponentially as in panels *a*–*c*. Cell divisions were set at fixed intervals coinciding with the average time to double cell volume; size variability was added by asymmetric divisions, using a standard deviation of 0.1 for cell division ratios (volume of each sibling cell relative to their combined volume) (107).

Erratum >

constant increment per unit of time (i.e., linearly), the faster relative growth of smaller cells and the slower relative growth of larger cells would automatically dampen variation, with no need to adjust cell cycle length to cell size (84) (**Figure 1**). This argument has been used to dismiss the need for cell size control in mammalian cells (20), although subsequent studies showed that at least some mammalian cell types have approximately exponential growth and consequently should have a mechanism to enforce size uniformity (42, 79, 88, 116).

Another reason for cell size control is that even if metabolic or geometric restrictions led to slower growth of larger cells (70, 77, 128), the resulting convergence of cell sizes would not necessarily lead to the optimal size for cell function (as discussed in more detail in Sections 8 and 9). In other words, sensing and correcting cell size may be required not only to ensure uniformity but also to optimize average size. As mentioned above, in multicellular organisms the average size of different cell types is influenced by extracellular signals (72). However, it remains unclear to what extent these signals interact with cell-autonomous mechanisms for size control inherited from unicellular ancestors. In mammalian cells, the evidence for cell-autonomous feedback between growth and cell cycle includes reduced variability at specific stages of the cell cycle (59, 111) and homeostatic recovery of cell sizes after experimental perturbation (26, 71). Similarly, plant cells adjust cell cycle progression to size at birth (55), and temporary perturbation of cell size was rapidly

**Exponential growth:** growth at a constant relative rate

corrected in subsequent cell cycles (107). Thus, feedback between cell size and cell cycle has been observed across a wide range of eukaryotes.

## 3. PHENOMENOLOGICAL MODELS FOR CELL SIZE CONTROL

Measurements of cell growth and cell cycle progression have been used not only as evidence that these processes are coordinated but as inferences about the underlying mechanism, which led to three main types of models (29, 80, 104) (**Figure 2**). In the timer model, cell division occurs at

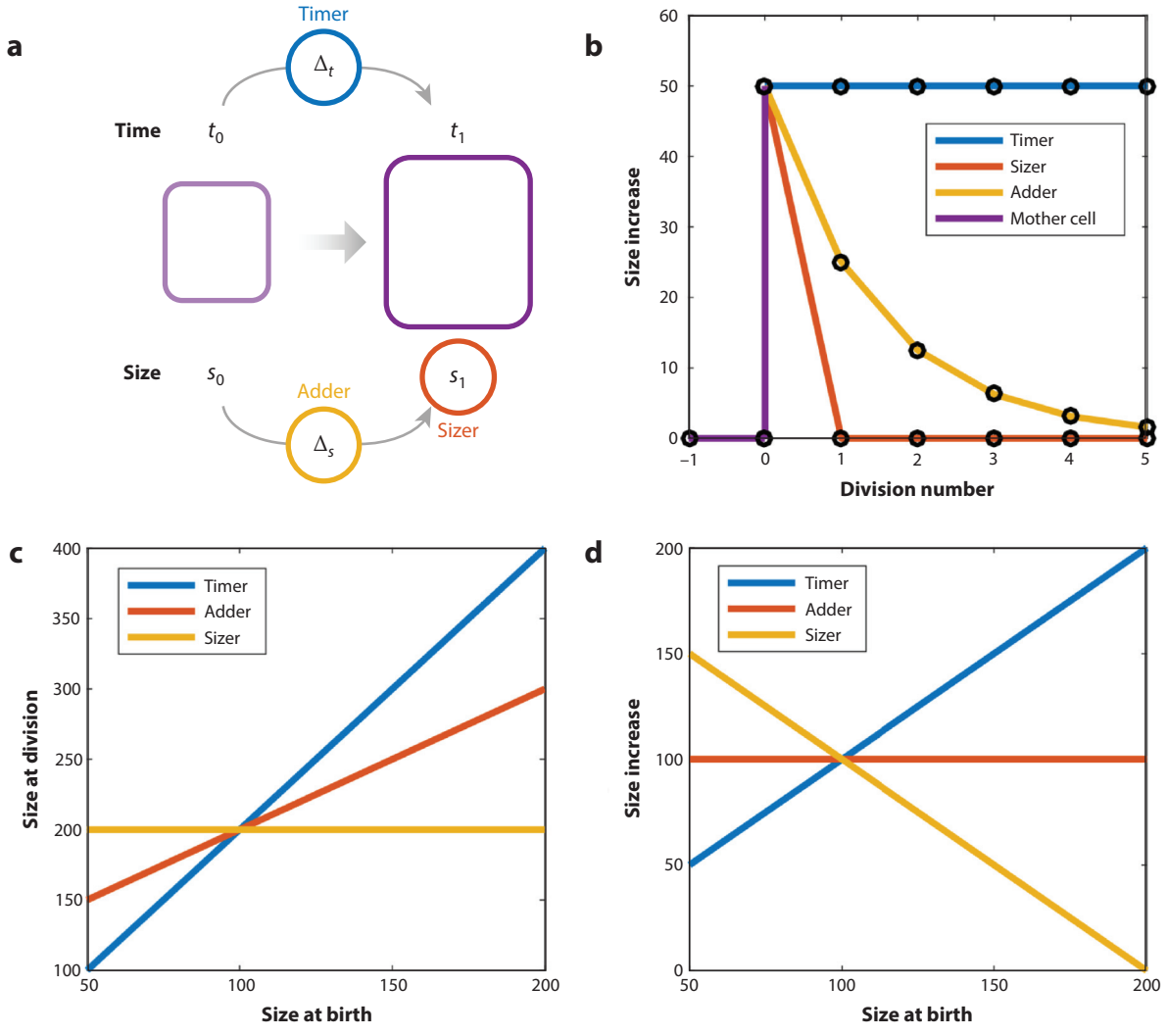


**Figure 2**

Phenomenological models of cell size control. (*a*) A cell (*purple*) growing from size $s_0$ at time $t_0$ to size $s_1$ in time $t_1$; the circles indicate the key parameters controlling cell cycle progression in each model: time interval for timers, size difference for adders, and final size for sizers. (*b*) Computer simulation of how a perturbation in cell size (indicated as percentage deviation from ideal size) propagates through cell divisions in each of the three models (cell growth was assumed to be exponential). (*c*,*d*) The three models predict different relations between cell size and division, illustrated by different slopes in plots of (*c*) size at cell birth versus cell size at division or (*d*) size increment from cell birth to division.

given time intervals with no feedback from cell size, so during exponential growth, the increase in size between cell birth and division is proportional to the initial cell size. In the sizer model, division is triggered at a given size threshold, so the increase is smaller for cells that are born large. By contrast, adder models assume a constant increase regardless of the initial size. The three models can be distinguished by plotting cell size increment from birth to division against cell size at birth: These plots are linear with slopes of −1 and 0 for sizers and adders, respectively, and +1 for timers, assuming exponential growth. These models also make different predictions about how cells respond to perturbations in their size: Timers perpetuate the perturbation, adders lead to a gradual return to normal size, and sizers correct size within one cell division (**Figure 2**).

In recent years, measurements of growth and cell cycle in individual cells have increased in number, precision, and temporal resolution. These measurements have often supported adder models, first in bacteria and subsequently in both yeast and mammalian cells (14, 15, 110, 120). However, apparent adder behavior can also result from the interaction between sizer and timer mechanisms working at different stages of the cell cycle (16). To resolve this issue, researchers must identify the molecular mechanisms. Multiple molecular models have been developed for sizers, as discussed in Section 4. For adders, a plausible theoretical mechanism not yet involving specific genes and molecules has been proposed (110).

The phenomenological models reviewed above have been discussed mostly in the context of size variability associated with exponential growth (i.e., assuming a constant relative growth rate). There is, however, the possibility that growth rate itself could respond to cell size (43), and recent evidence supports a role for cell cycle–dependent growth rates in mammalian cell size homeostasis (14, 41). Modulation of growth rate balanced with catabolism would also be relevant to the extensive regulation of cell size seen in differentiated cells that no longer divide (72), although there would be some caveats in implementing this mode of cell size control in plants, as discussed in Section 6.

**Phenomenological model:** directly represents the interactions between processes measured experimentally, rather than explaining these interactions as resulting from the behavior of components at smaller scales

**Relative growth rate:** increase in size over time expressed as the ratio between sizes at two successive time points

**Cyclin:** regulatory subunit of a kinase that controls progression through the cell cycle

## 4. MOLECULAR MODELS

### 4.1. Size-Dependent Transition to the DNA Replication Phase

One of the challenges in revealing molecular mechanisms for cell size control is to identify a structure or molecule that could be used as a ruler to measure cell size. Most organelles and the total amount of most proteins scale up with cell size (74, 106), so they would not be appropriate rulers. One obvious exception would be DNA, or specific sites on the genome. This idea has been a key part of models for the size-dependent progression from the gap 1 (G1) phase to the DNA synthesis (S) phase of the cell cycle.

In budding yeast cells, the G1 cyclin Cln3 phosphorylates Whi5 to derepress the SBF transcription factor, which activates transcription of genes required for S-phase entry. Cln3 synthesis mirrors overall protein synthesis rate and therefore cell growth (90). The fixed number of SBF-bound sites in the genome has been proposed to provide the ruler to measure Cln3 levels as a proxy for cell growth (121). In a more recent model, increasing cell volume dilutes the Whi5 protein to a threshold concentration that allows the G1-to-S transition (106) (**Figure 3*a***). In this case, Whi5 can function as the ruler because its synthesis rate is limited by gene copy number, not by protein synthesis capacity, which scales up with cell size, so the Whi5 dilution model indirectly uses the genome as the internal ruler.

In *Chlamydomonas reinhardtii*, a green alga distantly related to the unicellular ancestors of land plants, DNA replication is also linked to cell size. In this alga, a vegetative cell maintained in the light grows during a prolonged G1 phase to several times its initial size; shifting the cells to darkness stops growth, but if a size threshold has been passed, the cell undergoes a series of rapid

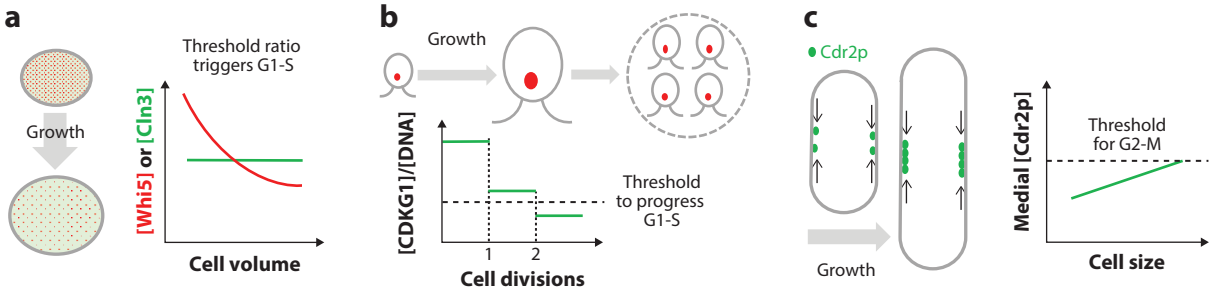


**Figure 3**

Molecular models for cell size control correspond to sizers that depend on dilution or accumulation of key cell cycle regulators. (*a*) In budding yeast cells, the G1-to-S transition is triggered when Whi5, which is diluted by cell growth, reaches a threshold ratio to Cln3, whose concentration remains constant (105). (*b*) The accumulation of the cyclin-dependent kinase CDKG1 by *Chlamydomonas reinhardtii* during an extended period of growth is followed by multiple rounds of division, whose number reflects the initial cell size because each round of DNA replication dilutes CDKG1 until a threshold level is reached (69). (*c*) In fission yeast cells, the Cdr2p kinase accumulates in the medial region of the cell in proportion to the cell's surface area, eventually reaching a threshold concentration that initiates the G2-to-M transition (87).

cycles through S and M phases (**Figure 3*b***). The number of these cycles is adjusted to the starting cell size through the retinoblastoma (RB) pathway (118), which is conserved between plants and mammals and functionally equivalent to the yeast Whi5 pathway (121). In *C. reinhardtii*, the cyclin-dependent kinase G1 (CDKG1) inactivates RB to promote S-phase entry; CDKG1 reaches a size-dependent concentration in the mother cell and is not produced during the subsequent divisions. Progressive dilution of CDKG1 in relation to its targets on increasing amounts of chromatin has been proposed to link the number of S/M cycles to mother cell size (69) (**Figure 3*b***). Accordingly, *cdkg1* mutants divide fewer times and produce larger daughter cells (69), whereas *rb* mutants divide too many times, resulting in small cells (118).

The control of cell division number in *C. reinhardtii* is reminiscent of early embryogenesis in many animals, which starts with a series of rapid cell divisions before the midblastula transition, when cell cycles lengthen and zygotic transcription increases (58). In *Xenopus laevis*, the number of rapid divisions has been proposed to depend on dilution of maternally deposited DNA replication factors (18, 19) or histones H3/H4 (2) against increasing amounts of DNA. Thus, as in *C. reinhardtii*, cell size and number at the midblastula transition are determined by the number of DNA replication cycles required to titrate an initial amount of a chromatin-interacting protein.

More recently, evidence has emerged that, similar to the role of Whi5 in budding yeast cells, dilution of RB links the G1-to-S transition to cell size in human cells (127). A key feature of the mechanism is that RB is synthesized mostly after G1, so most of the RB present during G1 is inherited from the mother cell and is diluted during growth. Consistent with a role for RB in the size-dependent G1-to-S transition, loss of RB function eliminated the negative correlation between size at birth and G1 length seen in wild-type cells. Together with the budding yeast and *C. reinhardtii* models, these results highlight an intimate connection between the Whi5/RB pathway and the size-dependent G1-to-S transition.

**Cyclin-dependent kinase (CDK):** protein kinase whose activity is enhanced by cyclins; phosphorylates many proteins involved in cell cycle progression

## 4.2. Mechanisms Linking Mitosis to Cell Size

In contrast to budding yeast and mammalian cells, the main target for cell size control in *Schizosaccharomyces pombe* is the transition from the second gap (G2) phase of the cell cycle to mitosis (M). In this case, different sizing mechanisms have been proposed, mostly converging on a series of kinases (Pom1, Cdr2, Wee1) that function sequentially to control cyclin-dependent kinase 1 (Cdk1),

which triggers the G2-to-M transition. Initially, activity of this pathway was proposed to reflect cell length through a gradient of Pom1 from the cell poles to the cell center, where Pom1 phosphorylates Cdr2. This was subsequently revised to a model in which Cdr2 accumulates in the medial region of the cell in a way that reflects cell surface area, eventually reaching a threshold that initiates the G2-to-M transition (87) (**Figure 3*c***). In a general sense, these models also rely on internal rulers that do not scale up in proportion to cell size, such as the source of Pom1 at the cell poles or the nodal band where Cdr2 accumulates.

An alternative recent model for the G2/M sizer in *S. pombe* is based on a size-dependent increase in concentration of the Cdc25 phosphatase, which dephosphorylates and activates Cdk1. In contrast to most other proteins, Cdc25 concentration increased with cell size, leading to a more rapid transition to mitosis in larger cells (62). As in the Whi5 dilution model in budding yeast cells (106) and the CDKG1 mechanism in *C. reinhardtii* (69), the Cdc25-based model relies on a cell cycle regulator whose total amount does not increase linearly with cell growth, although the mechanism behind the size-dependent increase in Cdc25 concentration remains unknown.

### 4.3. Complications: Overlapping Mechanisms and Cell-Type-Specific Features

Although supported by extensive evidence, the mechanisms described above remain debated because mutation of key genes such as *whi5* in budding yeast and *pom1* or *cdr2* in fission yeast does not eliminate cell size homeostasis. Robust cell size homeostasis has been suggested to result from the overlap of multiple size-sensing mechanisms at different stages of the cell cycle. For example, cell size uniformity in *Saccharomyces cerevisiae* depends not only on the well-studied G1/S sizer but also on the less well-characterized size control at the G2-to-M transition (40). Similarly, *S. pombe* too has a G1/S sizing mechanism, which becomes visible only when the G2/M size control is compromised in *wee* mutants (35, 125). In the *S. pombe cdr2* mutant, the area-sensing mechanism shown in **Figure 3*c*** appears to revert to a secondary, volume-sensing mechanism (30). In plants, there is evidence that both the G1-to-S and the G2-to-M transitions are responsive to cell size (55). That more than one pathway exerts control in more than one cell cycle stage complicates the analysis of overall cell size homeostasis.

In summary, the molecular mechanisms for cell size regulation remain an area of active investigation in all eukaryotic models. The mechanisms proposed converge on core regulators of the G1-to-S transition (Whi5/RB) or on the mitotic Cdk to regulate the G2-to-M transition. How information about cell size is imparted to these cell cycle regulators, however, may be organism specific and influenced by features such as cell geometry (as in the case of fission yeast, which elongate while maintaining the dimensions of the poles and medial region) or life cycle (as in the case of *C. reinhardtii*, with its prolonged growth followed by rapid sequential divisions). Therefore, to understand cell size regulation in multicellular plants, we should consider specific features of plant cell growth, as discussed in Section 6.

## 5. RELATION BETWEEN PLOIDY AND CELL SIZE

The hypothesis that DNA could be used as a molecular ruler in cell size homeostasis also suggests a link to the observation that cell size correlates with ploidy levels in a wide variety of eukaryotes, including plants. Although this phenomenon has been known for over a century (9), its molecular basis has remained as elusive as the mechanism for cell size control.

On the basis of the observation that nuclear and cytoplasmic volumes remain proportional for each cell type, and of the expectation that nuclear size depends on chromatin content, researchers have hypothesized that ploidy sets nuclear size, which then determines overall cell size (9, 17, 112). However, the available data show that although DNA content may impose a minimum nuclear

volume, the cytoplasm has a predominant role in the control of nuclear size, likely through the control of nuclear import (67). Accordingly, DNA replication does not increase nuclear size in budding yeast cells (57), and in mammalian cells with DNA replication blocked with aphidicolin, nuclear size continued to increase with cell growth (41). In *Arabidopsis* sepals, the *crowded nuclei 1* (*crwn1*) mutation, which affects a nuclear matrix constituent protein and results in small, dense nuclei (98, 122), did not alter cell volumes in diploid or polyploid cells, indicating that nuclear volume does not mediate the effect of chromatin contents on cell size (92).

A second hypothesis is that DNA sets an upper limit for biosynthetic activity, either through overall transcription or by limiting ribosome biogenesis (112). Increased ploidy can result in a proportional increase in transcription per cell, as seen in tomato fruits with high levels of ploidy (8). Furthermore, mathematical modeling (70) and evidence from fission yeast cells (128) supported the idea that as cells enlarge, DNA template can eventually become limiting for transcription rates and consequently for the cellular growth rate. However, both fission yeast and mammalian cells normally maintain sizes at which the transcription rate is limited not by the amount of DNA template, but by the concentration of the gene expression machinery (86, 128). In the *Arabidopsis* meristem, transient overexpression of the CDK inhibitor KRP4 (KIP-RELATED PROTEIN 4) resulted in cells up to 10 times larger, which recovered their normal diploid cell volume when the cell cycle inhibition was lifted; throughout the experiment, cellular growth rates were comparable to those of normal-sized cells, showing that the diploid nuclei could still sustain growth of a much larger cytoplasmic volume (107). Thus, within the physiological range, it is not obvious that cell size is limited by the concentration of DNA template.

A third hypothesis is that polyploid cells are larger because DNA is used as a ruler to gauge cell size (3). As mentioned above, entry into S phase in budding yeast cells is triggered by dilution of Whi5, whose synthesis rate is proportional to gene copy number rather than cell size (106). In diploid yeast cells, which are approximately twice the size of haploid cells, mutation of one Whi5 copy returned the cells to a haploid size, whereas adding an extra copy of Whi5 made haploid cells as large as the diploids, supporting the idea that Whi5 copy number mediates the effect of ploidy on cell size (106). In both the Whi5 model and a previous budding yeast model based on titration of Cln3 against SBF bound to S-phase genes (121), the effect of ploidy on cell size would be mediated through the copy number of specific loci. In this scenario, aneuploidy would have differential effects on cell size, depending on which chromosome is affected. Plants are particularly tolerant of aneuploidy and therefore would be useful for testing this prediction. *Arabidopsis* aneuploid lines show different growth phenotypes associated with copy number of specific chromosomes, including meristem-related defects (49); it would be interesting to extend this analysis to meristem cell size.

At the same time, cell size correlates positively with bulk genome size across plants, even after the effects of ploidy are excluded (5), a trend also seen in animals (46). Cell size differences correlated primarily with the accumulation of transposable elements would be easier to reconcile with a mechanism that senses bulk DNA rather than loci with specific roles in cell cycle progression. The titration of histones during early *X. laevis* development (2) offers a precedent for how bulk cellular DNA could be gauged, although there is currently no link between general chromatin-binding proteins and cell size homeostasis.

## 6. SPECIFIC FEATURES OF PLANT CELLS AND CONSEQUENCES FOR SIZE REGULATION

Conserved cell cycle regulators may be linked to diverse aspects of cell size in different cell types and species. A defining feature of plant cells is that they are encased in cell walls, which constrain

growth and prevent movement relative to adjacent cells (66). To grow, plant cells must push against their own walls and those of their neighbors. The required force comes from turgor pressure, and the rate of growth, if defined as increased physical dimensions, depends on how easily the wall yields to pressure; not surprisingly, the mechanical properties of plant cell walls are subject to extensive biochemical and genetic control (11, 22). The increased volume created by wall extension is occupied by enlargement of the cytoplasm and nucleus or by water uptake and expansion of vacuoles, with the balance of the two depending on cell type and developmental stage (**Figure 4**). Cell expansion associated with vacuole enlargement probably evolved as a metabolically low-cost mechanism for rapid and extensive organ growth under competition for light and other environmental resources.

**Turgor pressure:** force that results from the osmotic flow of water into the cell and pushes the plasma membrane against the cell wall

The specific features of plant cell growth have implications for the control of cell size. First, the increase in physical dimensions of plant cells cannot usually be reversed, in contrast to postmitotic

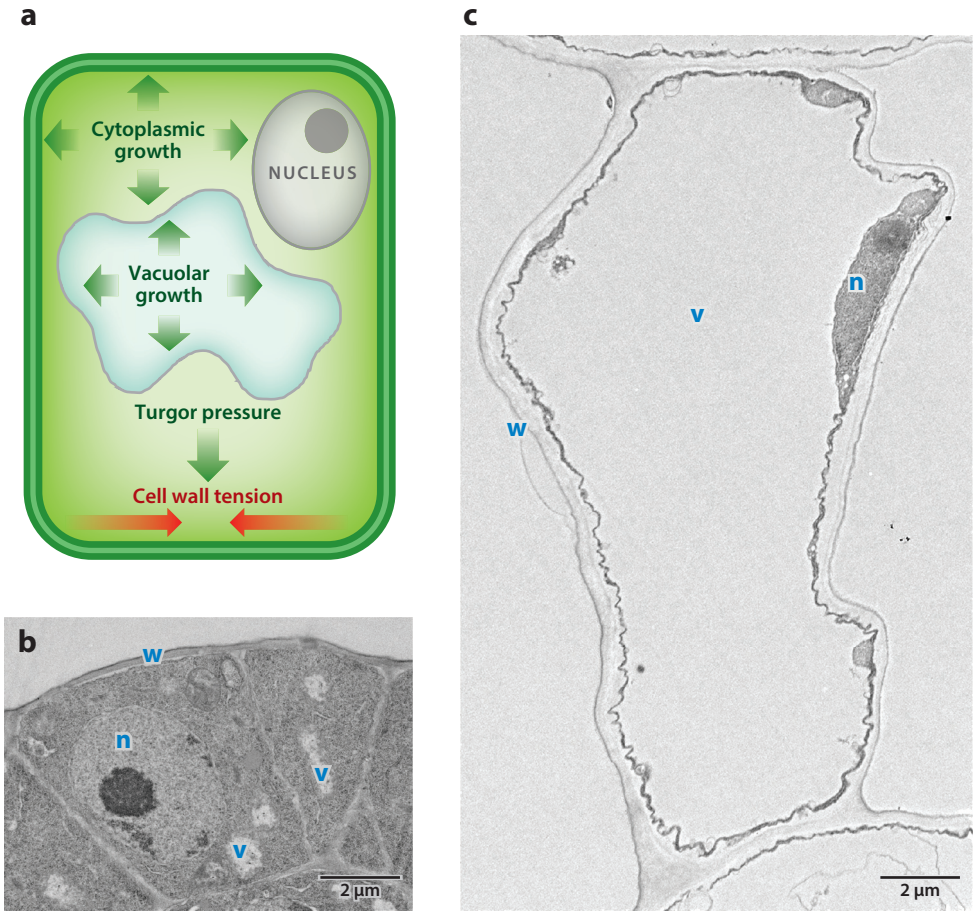


**Figure 4**

Plant cells increase in size by turgor-driven wall extension combined with cytoplasmic growth and developmentally regulated vacuole expansion. (*a*) The growth-constraining effect of cell walls (*red arrows*) and growth-promoting processes (*green arrows*). (*b*,*c*) Electron micrographs of cells from the *Arabidopsis* shoot meristem (*b*) and the lower leaf epidermis (*c*), with the nucleus (n), cell wall (w), and vacuoles (v) indicated; note the large proportion of the cell volume occupied by the vacuole in the differentiated leaf cell.

animal cells, whose size depends on the dynamic balance between biosynthesis and degradation (72). The space enclosed by the cell walls does not normally shrink, although there are exceptions such as the stomatal guard cells, whose volume changes reversibly to open and close epidermal pores for gas exchange (114). Modulation of guard cell volume is associated with changes in vacuole structure and size (39). This dynamic nature of vacuoles (13) and their role in autophagy (60) make it likely that, although encased within irreversibly grown walls, the cytoplasmic volume is in equilibrium between biosynthesis and catabolism, with oscillations in volume balanced by vacuolar changes. Accordingly, the auxin hormone has been proposed to inhibit cell expansion by restricting vacuolar changes required to maintain cytosol homeostasis (102).

Second, the role of vacuoles in cell expansion can decouple cell dimensions from cytoplasmic synthesis. Because dimensions and protein content are not necessarily proxies for each other, it is important to define precisely which could be under homeostatic control and linked to cell cycle progression. This matters not only to reveal molecular mechanisms but also to understand the interaction between size control in individual cells and across tissues. On the one hand, if size is defined as cell dimensions, individual growth rates should be constrained by the physical connections with neighboring cells; therefore, plant cells would have the freedom to maintain size homeostasis only by adjusting cell cycle to size, in contrast to the recent evidence that mammalian cells regulate individual growth rates to maintain size homeostasis (14, 41). On the other hand, if growth is defined as macromolecular synthesis, the oscillations in vacuole size could accommodate temporary discrepancies between cell-autonomous growth control and physical constraints on cell dimensions imposed by interconnected cell walls.

Third, growth constrained by cell walls is associated with a specialized mode of cell division that is also relevant to cell size control. Plant cells divide by building new walls that connect with preexisting walls. The plane of cell division is believed to be defined by a microtubule array that radiates from the nucleus to the cell cortex and settles on a stable configuration that bridges the nucleus to the nearest cell walls (73). While this mechanism would tend to produce equal divisions, the actual planes of cell division show considerable variability (107, 108). Researchers have proposed that in any given cell, the final division plane is set by competition between alternative configurations with local energy minima, which depend on cell shape (6) and are expected to be influenced by mechanical stress, which affects the dynamics of microtubule arrays (31, 100). Thus, the way plant cells divide, combined with the physical constraints due to interconnected cells, may impose asymmetries in cell division that would need to be corrected if uniform cell sizes are to be maintained.

## 7. CELL SIZE CONTROL DURING PLANT DEVELOPMENT

### 7.1. Cell Size Control in the Meristem

As in all multicellular organisms, autonomous control of cell size in plants would have to be integrated with developmental controls that coordinate tissue growth and produce specialized cell types. Plants are convenient for addressing this issue because organ initiation, patterning, and differentiation occur continuously at their growing apices and are not limited to embryogenesis. Development of new plant organs and tissues begins at the apical meristems, where stem cell niches sustain the continuous initiation of organ primordia (44). Meristem cells maintain uniform sizes over extended periods of proliferation, do not endoreduplicate, and have small vacuoles (65, 91) (**Figure 4**), simplifying the analysis of how the cell cycle relates to cell dimensions (**Figure 5**).

To account for stable cell sizes, spatial models of meristem development have typically assumed that individual cells divide at a constant size, twice their average birth volume (i.e., using a perfect sizer) (27, 56, 109). However, the question of how meristems maintain uniform cell sizes has only

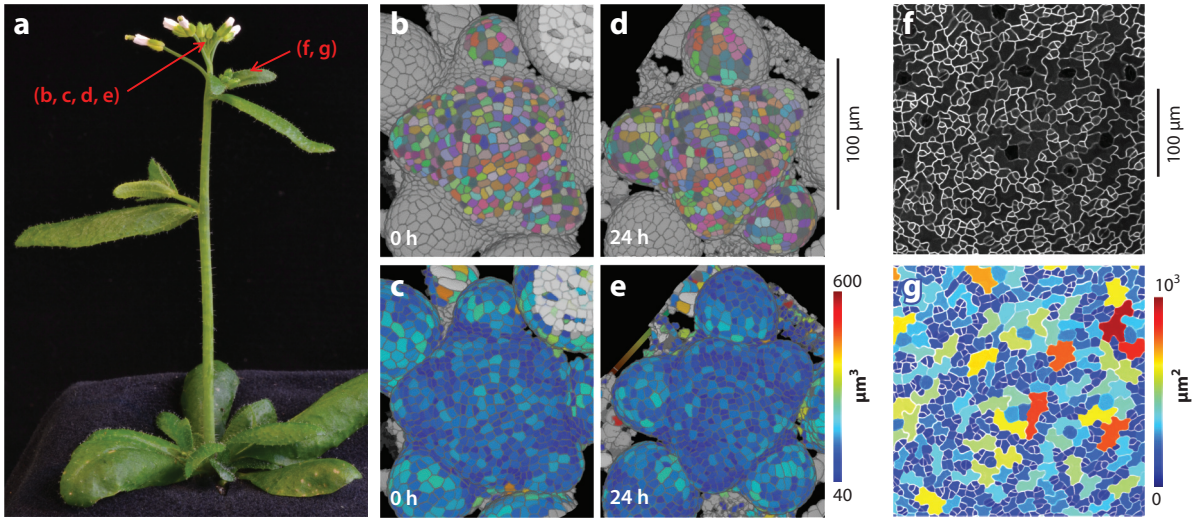


**Figure 5**

Live cell imaging and quantitative analysis of cell growth and division in plant tissues, illustrating uniform cell sizes maintained in the apical meristem and cell size heterogeneity during organ development. (*a*) *Arabidopsis* at the flowering stage, with arrows pointing at the location of tissues shown in panels *b*–*g*. (*b*–*e*) Three-dimensional views of segmented confocal image stacks of the same shoot apical meristem at an initial time point (*b*,*c*) and 24 h later (*d*,*e*). Cells in panel *b* and their descendants in panel *d* are marked in the same color. In panels *c* and *e*, cells are colored by volume (scale next to panel *e*). The scale bar next to panel *d* applies to panels *b*–*e*. (*f*,*g*) Live cell image of young leaf epidermis (*f*) and corresponding segmented image with cells colored by area (scale bar next to panel *g*); the scale bar next to panel *f* applies to panels *f* and *g*.

recently been addressed experimentally (55, 107, 123). The first indication that meristem cells coordinate cell cycle progression with their size came from time-lapse 3D imaging combined with computer simulations, which suggested that feedback between growth and cell cycle in individual cells would be necessary to counteract the size variability introduced by asymmetric divisions and variable cellular growth rates (107). A feedback between cell size and cell division was also revealed by experiments following recovery of cell size after genetic perturbation (107). More detailed measurements of growth and division times over several generations supported cell-autonomous size control (123). However, plots of size increase as a function of birth size were not consistent with any of the main phenomenological models (timer, sizer, or adder), suggesting a more complex mode of regulation (123) that could result from overlap of multiple controls at different cell cycle phases, as mentioned above for yeast and mammalian cells (14, 40, 120).

A more recent study confirmed that progression through the cell cycle is linked to meristem cell size at both the G1-to-S and the G2-to-M transitions (55). A model was proposed in which CDK accumulated at a rate proportional to cell size (i.e., using the production rate of cell cycle regulators as a proxy for cell growth, as in previous yeast models) (90). The model could account for stable meristem cell sizes and for the increase in average cell sizes seen in *cdkb1* mutants and the decrease seen in *cyclin D3* mutants (55); however, it remains unclear how meristem cells would assess their total level of CDK activity against an internal ruler.

**Cell cycle phase:** proliferating eukaryotic cells cycle through phases termed G1 (gap 1), S (DNA synthesis), G2 (gap 2), and M (mitosis)

## 7.2. Cell Size During Organogenesis

After cells are recruited away from the meristem and into organ primordia, they divide a limited number of times before differentiating into cell types with different shapes and sizes (**Figure 5**).

Changes in growth rate, in cell size, and in the correlation between cell volume and cell cycle phase are seen shortly after primordium initiation (103). Some of these changes reflect a rapid increase in vacuole volume, resulting from inflation of the highly branched, tubular vacuoles of meristem cells. The immature tubular structure with a high surface-to-volume (SV) ratio might ensure that there is enough vacuolar membrane available to sustain the rapid increase in vacuole size during organogenesis (65).

**Surface-to-volume (SV) ratio:** the surface area divided by the volume of an object

**Endoreduplication:** modified cell cycle in which mitosis is skipped, resulting in a single cell with twice the initial DNA content

As mentioned above, the onset of vacuolar growth has implications for cell size control. If cell cycle progression depended on geometric features such as cell surface area or volume, the threshold values would have to increase in proliferating cells that also show vacuolar growth. If control is exerted on cytoplasmic volume or a connected variable, such as protein synthesis rate, then it remains to be seen whether these features remain under homeostatic control in vacuolating cells. Unfortunately, the contribution of these processes to cell growth cannot be genetically separated because the vacuole also has vital functions in ion homeostasis, detoxification, and protein storage. Thus, mutants that are unable to form the vacuole are embryo lethal (e.g., *vacuoleless* mutants, which disrupt a conserved pathway for docking and fusion of tonoplast vesicles) (95).

Vacuolar expansion continues after cell division ceases and can lead to dramatic increases in cell dimensions (112). It remains unclear how the rate and duration of cell expansion are controlled and to what extent this control is imposed at the tissue level or involves cell-autonomous decisions. Growing roots show a dynamic equilibrium between regions of cell proliferation (meristem), expansion (with no division), and final differentiation (with no further growth), and the dynamics of these regions has been modeled to address how cell expansion is terminated (89). Three models were considered: ruler, in which expansion stops at a distance from the meristem; timer, in which cells expand for a given time; and sizer, in which cells expand to a target size (note that despite similar names, these models should not be confused with the models shown in **Figure 2**). These models make different predictions; for example, the ruler predicts a fixed size of the expansion region, whereas the sizer and timer predict that the expansion region would increase and decrease, respectively, with reduced cell elongation rate. Measurements of the natural variability between wild-type roots or of variability caused by changes in hormone signaling better matched the sizer model (89). This raises the question whether a mechanism that gauges plant cell dimensions could inform not only cell cycle progression but also cell expansion, although identifying molecular mechanisms remains a key challenge.

In addition to vacuolar expansion, increased ploidy through endoreduplication contributes to cell enlargement during organ development (12). This has been well documented in the leaf and stem epidermis of *Arabidopsis* (75), in the development of trichomes (hairs on the shoot epidermis) (53), and in the development of giant cells in the sepals of *Arabidopsis* flowers (93). In both trichomes and sepal giant cells, the distribution of polyploid cells within the tissue is believed to result from the reinforcement of initially random fluctuations in the expression of transcription factors, which subsequently activate CDK inhibitors to promote the shift from mitotic cycles to endoreduplication (53, 76). In the epidermis, final cell size correlates with the number of endocycles, which in turn depends on the timing of the transition to endoreduplication (12, 93). The effect of ploidy on cell size has been proposed to vary between tissues (61), although a clear interpretation of these results depends on separating the cytoplasmic and vacuolar components of cell size.

In summary, there is evidence that uniform cell size is maintained by feedback between cell size and cell cycle progression in meristems, although the molecular mechanism remains unknown. In developing organs, endoreduplication and vacuolization contribute to larger and more heterogeneous cell sizes. However, it remains unknown whether cell size sensing and responses are absent during organogenesis or modified in different cell types.

## 8. RELEVANCE OF SIZE FOR CELLULAR FUNCTION

An underlying assumption in cell size regulation is that size has consequences for the fitness of individual cells or of the whole organism. In individual cells, one likely reason for this is metabolic allometry; that is, metabolic rates do not scale linearly with cell size (78). In particular, Miettinen & Björklund (77) proposed that mammalian cell size is adjusted for optimal mitochondrial dynamics and function. Multiple reasons for metabolic allometry have been suggested, including changes in intracellular distances and in the SV ratio, which is expected to affect nutrient import, waste removal, and the control of intracellular ion concentrations (78). The effects of cell size on SV ratio and on distances to the cell surface, however, might be counteracted by changes in cell shape (more relevant in motile animal cells) and, in the case of plant cells, by vacuolar changes (i.e., modulating cytoplasmic volume without changing cell surface area).

**Metabolic allometry:** property of changes in metabolic activity that do not scale in proportion to the size of a cell or organism

**Finite element method:** used to solve complex problems in physics and engineering, by subdividing a continuous system into discrete parts called finite elements

In budding yeast cells, the change in SV ratio for cells of different size (but same shape) has been linked to specific transcriptional responses: Cell size changes due to ploidy or *cln3* mutations were associated with altered expression of genes with functions relevant to the cell surface functions (cell wall, extracellular matrix, and plasma membrane) (126). In plants, there have been extensive studies of transcriptome changes associated with polyploidization. Unfortunately, it is difficult to infer gene expression changes associated specifically with cell size because these studies typically have compared whole plants of organs with the consequent cell type heterogeneity and physiological effects (24). Recently, however, an extensive analysis of ploidy, cell type, and gene expression in *Arabidopsis* roots revealed that increased ploidy was associated with changes in expression of genes associated with chromatin functions, ion transport, and cell wall modification (7). As in budding yeast, genes related to cell wall modification are relevant to cell surface functions and may have a role in adjusting cell wall mechanics to cell size (7) (see Section 9).

If the expression of surface proteins responds to cell size, then so could other relevant genes, for example, involved in cell differentiation. This has been documented in the alga *Volvox carteri*, in which embryonic cells undergo a series of asymmetric divisions, at the end of which a colony differentiates, with large germ cells (gonidia) surrounded by small, flagellated somatic cells. It had been hypothesized that these cell types are specified by asymmetric segregation of differentiation factors; however, perturbation of cell division planes and mutants with premature arrest of cell divisions showed that cell fate depended instead on size, with any cell larger than 8 μm in diameter differentiating as a gonidium (63). Size-dependent cell fate has not been shown as clearly in higher plants, but there is evidence that endoreduplication, with the associated increase in cell size, is required to maintain cell fate in *Arabidopsis*. Although both trichomes and giant sepal cells establish their identity before the commitment to endoreduplication, cells that fail to endoreduplicate cannot maintain their identity and revert to the default epidermal fate (defined by morphology and gene expression), as seen in sepals mutant for the CDK inhibitor LGO (LOSS OF GIANT CELLS FROM ORGANS) or after ectopic expression of the CDK inhibitor KRP1 in early trichomes (10, 94).

Cell size and shape are also expected to affect a cell's mechanical properties, which play a central role in plant morphogenesis. The effect of cell size has been supported by finite element method–based models, in which larger cells expanded more than smaller cells, assuming equal turgor pressure and the same cell wall properties (4). A higher tensile stress in the walls of larger cells is also consistent with developing trichomes protruding from the surrounding epidermis; in mutants in which the developing trichome divides instead of growing as a single large cell, bulging is inhibited (10, 50). Similarly, sepal epidermal cells that increase in size through endoreduplication bulge out of the epidermis (93).

## 9. REPERCUSSIONS OF CELL SIZE ACROSS SCALES

Beyond local effects on metabolism, differentiation, and cell mechanics, cell size has consequences at the organ and organismal levels. In plants, one reason for this is the propagation of mechanical stress across connected cells (50, 93). Simulations of stress patterns in growing plant tissues demonstrated that the placement of new walls by cell divisions can reduce tissue stress and that the orientation of divisions can affect growth heterogeneity and tissue shape (1). The local buildup of mechanical stress during cell expansion can also be accommodated and reduced through changes in cell shape, as proposed for the development of puzzle-shaped leaf epidermal cells (101).

The increased stress on the walls of larger cells implies that delayed division or a shift to endoreduplication should lead to changes in stress patterns and consequently in organ shape. For example, the sepal giant cells are suggested to generate tension abaxially, causing the sepals to curve outward upon flower maturation; supporting this idea, sepals with supernumerary giant cells curved outward prematurely, whereas mutants lacking giant cells remained bent inward even after flower opening (93). Ironically, in mutants with impaired ability to adjust cell wall extension to mechanical stress, local growth becomes more uniform but organ shape more variable (as seen in meristems with microtubule dynamics disrupted by a katanin mutation or in sepals with abnormal production of reactive oxygen species due to mutation of the *FTSH protease 4* gene) (52, 119). The likely reason for this is that heterogeneity in cell growth is necessary to accommodate local mechanical stress in growing tissues, avoiding consequences at the organ scale.

In addition to affecting tissue mechanics, cell size can have a cumulative effect on organ dimensions. This is expected in structures made of a determined number of cells, as in the nematode *Caenorhabditis elegans* (21). However, differences in cell size can also be accommodated by compensatory changes in cell number to maintain organ and organism size, as shown by classical studies of salamanders with different ploidy (32) or seen in *Drosophila* wing development with altered expression of cell cycle regulators (82). In plants, the cumulative effect of cell size depends on the tissue and organ. The larger cells of tetraploid *Arabidopsis* form proportionately larger meristems (**Figure 6**), whereas sepals do not enlarge to the same extent (92) and leaf size does not change

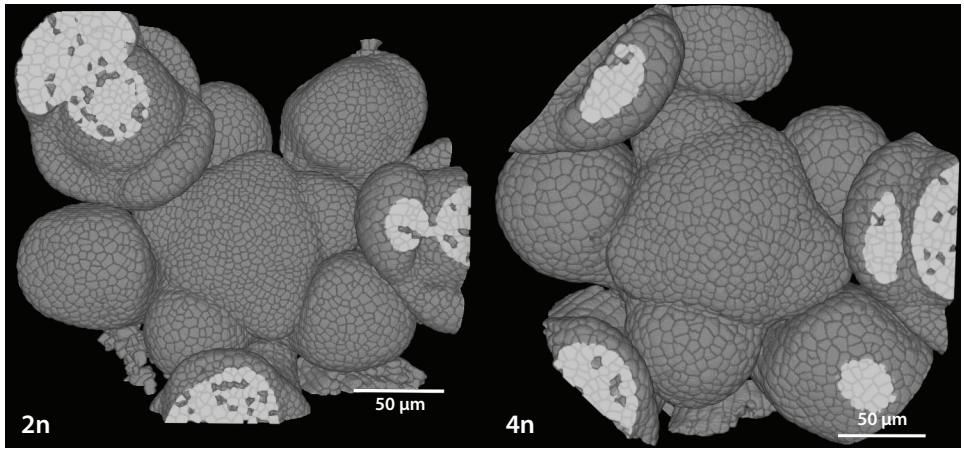


**Figure 6**

Polyploidy leads to increased cell and organ size in the *Arabidopsis* shoot apex. Three-dimensional reconstructions from confocal image stacks of isogenic diploid (2n) and tetraploid (4n) shoot apices; meristem size increases in proportion to cell size in the tetraploid (imaging and image analysis as in Reference 103).

(23). It remains to be seen whether these organ-specific responses to ploidy relate to the developmental changes in cell growth discussed in Section 7. The relation between ploidy, cell size, and organ size has practical importance in plants: Genome size and consequently cell size correlate positively with seed mass (64), which has been proposed as a reason why polyploidy has been important in the domestication of crop species (99).

Leaf size is also resilient to early changes in cell proliferation, which are compensated by the degree of subsequent cell expansion (36, 48, 51). The simplest explanation for the compensation between cell size and cell numbers in leaves is that cessation of leaf growth, whether by proliferation or cell expansion, is signaled at the organ level. The implications of this for cell size control depend on how size is assessed. If the relevant parameter were cell geometry, then cell-autonomous size control in leaves would have to be either absent or overridden by organ-wide signaling. Alternatively, cytoplasmic volume or the ratio of cytoplasm to DNA content could remain under cell-autonomous control in leaves, while superimposed vacuole expansion would adjust cell dimensions to a target organ size. As discussed above, resolving this issue requires disentangling the roles of cytoplasmic and vacuolar growth in plant cell size regulation.

Another consequence of cell size for organogenesis arises from the fact that cells, as units of gene expression, limit the resolution that can be achieved when establishing expression patterns. Accordingly, manipulation of cell sizes in *Arabidopsis* floral buds resulted in irregular expression of organ boundary genes and changes in organ number (107). The scaling between cell and organ sizes also has developmental consequences in amphibians, in which larger genome and cell sizes are associated with reduced complexity of the central nervous system (96). This could result simply from the lower number of building blocks or from an increase in cell cycle length, leading to a retardation of brain development and consequently to simplified structures (68, 96). In angiosperms, too, cell cycle length correlates positively with the size of their haploid genome; that is, larger genomes due to buildup of transposons take longer to replicate, although polyploidy does not necessarily slow down the cell cycle likely because replication origins also increase in number (37). The effect of genome size on cell cycle length may constrain the overall life cycle: Plants with larger genomes tend to have longer life cycles (i.e., are more often perennials than annuals) (64).

The importance of size for the function of particular cell types can also have selectable consequences at the organismal level. Stomatal guard cells, whose dimension is a strong predictor of genome size and affects the efficiency of gas exchange, are a good example in plants (5, 38). Considering the fossil record and the physics of gas exchange through pores, decreased size and increased density of stomata have been suggested to play an important role in plant adaptation to the gradual fall in carbon dioxide levels over the last 400 million years (38). In mammals, the need for efficient gas exchange has been proposed as a constraint on the size of blood cells and consequently on the evolution of genome size (45). Another example of the relevance of cell size over long evolutionary timescales is the suggestion that the metabolic advantages of smaller cells played an important role in the evolution of avian genomes from dinosaur ancestors (85). Thus, in both animals and plants, changes in cell size are a plausible link between genome evolution and selective pressures that operate on organismal features.

## 10. CONCLUSIONS AND PERSPECTIVES

Cell size control remains one of the fundamental riddles in biology. Links between cell cycle progression and cell size, initially established in yeast cells, have now been shown in a wide range of eukaryotes, plants included. The underlying molecular mechanisms, however, remain debated and, in the case of plants, unknown. Common themes have emerged across kingdoms, for example, the widespread role of the Whi5/RB pathways in cell size–dependent progression to S phase. At the

same time, there are organism-specific features of cell size control. A main challenge for the future will be to reveal the molecular mechanisms of cell size regulation in plants, the extent to which they include conserved strategies and genetic pathways, and how they relate to plant-specific features such as vacuolar growth and growth constrained by interconnected cell walls. Plant-specific aspects may also reveal unique strategies to implement cell size control in a multicellular context, for example, the potential role of vacuolar growth to resolve conflicts between growth regulation at the cellular and organ scales. Clarifying how cell size control relates to ploidy-dependent cell size will be especially important in plants: Ultimately, this will help us understand how cell size is linked to genome evolution and to how plants, including our crops, grow.

## DISCLOSURE STATEMENT

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