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Opinion

Double or Nothing? Cell Division and Cell Size Control

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Size is a fundamental property that must be tightly regulated to ensure that cells and tissues function efficiently. Dynamic size control allows unicellular organisms to adapt to environmental changes, but cell size is also integral to multicellular development, affecting tissue size and structure. Despite clear evidence for homeostatic cell size maintenance, we are only now beginning to understand cell size regulation in the actively dividing meristematic tissues of higher plants. We discuss here how coupled advances in live cell imaging and modelling are uncovering dynamic mechanisms for size control mediated at the cellular level. We argue that integrated models of cell growth and division will be necessary to predict cell size and fully understand multicellular growth and development.

Highlights

Size is fundamental to the structure and function of cells and tissues.

To maintain cell size in populations of actively dividing cells, a careful balance between cell growth and division must be established.

Advances in live cell imaging and lineage tracking, together with computational modelling, are allowing hypotheses based on the behaviour of unicellular organisms to be tested in multicellular tissues of higher plants.

Cell size homeostasis as observed at the tissue level appears to be an emergent property of a dynamic system that links cell growth, cell size, and cell-cycle progression at the level of individual cells, and is dependent on environmental and developmental conditions.

Size Control and Cell Division

The cell is the basic unit of life: a contained space that isolates reactions from the surrounding environment and forms the basic building block of tissue structure. The evolution of cells is thought to have improved the efficiency of essential processes, but the success of this strategy is dependent on size [1]: a small cell may have limited biosynthetic capacity or fail to assemble intracellular structures, but a large cell may be limited by the rate at which molecules are able to diffuse across it. Size can also affect the physical properties of the cell, such as strength and extensibility [2], which may impact on tissue growth. Size may also affect transmission of signalling molecules and specialised functions such as those of stomata [3]. Optimum cell size therefore varies according to species, cell type, and environment, and must be tightly regulated.

Various strategies could control size during postmitotic growth [4], but regulation of cell size becomes more challenging if cells are also actively dividing. Each division removes a large cell from the population and replaces it with two smaller cells, and therefore cells must on average double in size before dividing if cell size is to be maintained. Our best understanding of how growth and division are balanced comes from unicellular organisms [5], in which cell-autonomous mechanisms regulate the progression of the cell cycle through a series of cell size checkpoints. Although active at the level of individual cells, these dynamic mechanisms result in the emergence of coordinated behaviours that can be observed at the population level and allow cell size to respond to changes in environmental conditions.

Many growing multicellular tissues are also composed of populations of actively dividing cells, but much less is known about cell size regulation in these contexts. In plants, compensatory trade-offs between cell size and cell numbers mean that changes in cell size often have no on effect overall organ size [6,7]. One interpretation of this observation is that tissue growth is the primary target of regulation, and cell division plays an essentially passive role in subdividing the resulting volume according to fixed rules. However, new studies are showing that such responses may in fact be the result of much more dynamic cell size control mechanisms that operate at the level of individual cells, and that may play a significant role in shaping the structure of the tissue. We review here how time-lapse imaging, lineage tracking, and mathematical modelling are being used to uncover dynamic, cell-level behaviours in plant **meristematic tissues** (see Glossary), and we discuss how these cell size control mechanisms may contribute to multicellular processes such as tissue growth and patterning.

Cell Size Is Regulated at the Level of the Cell

Several mechanisms for cell size control in mitotically active populations have been proposed (Figure 1) [8]. Assuming that all cells within a tissue grow at a constant rate, and divisions are symmetric, cell size can

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be maintained using a molecular clock that produces a regular cell-cycle length (Figure 1A). If cycle length is equal to the time required for cells to double in size then a stable distribution of cell sizes will be produced, but, if not, this mechanism can lead to an increasing degree of variation [9]. Nonlinear growth coupled with cell-to-cell variation makes this type of system insufficient to regulate cell size in unicellular organisms [5,10], but the evolution of tissue-level signals in multicellular organisms might provide sufficient coordination between cells to make a timer mechanism feasible. Evidence from animal cells is conflicting [10], but the presence of shared walls between plant cells could further improve coordination in plant tissues by providing mechanical constraints to growth and prevent migration.

In support of the tissue-level hypothesis of cell size control, classic experiments identified that plant cells are dependent on growth hormones and mitogens to sustain population growth in culture: auxin for growth and cytokinin for division [11]. These hormones are also essential for establishing and maintaining actively dividing stem cell pools *in planta*, and extensive crosstalk ensures that their antagonistic activities are balanced [12,13]. Despite this regulation, many studies have shown that growth rates vary considerably from cell to cell, even between neighbours [14–20]. Furthermore, the geometric and topological rules that govern division-plane orientation mean that cells do not always divide symmetrically, and daughter cells of unequal sizes are frequently produced [21–23]. Multiple recent models agree that, when these sources of cell-to-cell variation are included in simulations of plant cell size [15,16,18], timer models using fixed cycle lengths are insufficient to maintain the constant distribution of cell sizes seen in the **shoot apical meristem (SAM)**. In fact, a timer mechanism might be used to amplify differences in cell size in the developing leaf epidermis [19]. The failure of these simulations to generate stable cell sizes over time demonstrates that, even though tissue-level signals may establish a broad coordination between growth and division, a mechanism is still necessary to remove variation in size at the level of the individual cell (Figure 2).

To understand better how cell size is regulated, live cell imaging and lineage tracking have been used to follow growth and division of individual cells (Box 1). Serrano-Mislata and colleagues created abnormally large SAM cells by transiently inducing a block in the cell cycle [15]. When this block was removed, the cells divided more rapidly than normal and returned towards a smaller size. Similar corrective cycles are also observed when large cells are generated through unequal divisions [16,18], suggesting that cell-cycle length is not fixed, but instead is variable and acts to maintain cell size close to the population average. In contrast to timer models, models including this corrective, inverse relationship between cell size and cell-cycle length are sufficient to maintain a constant cell size distribution [16,18]. This demonstrates that the behaviour of individual cells is significant to the coordinated behaviour of the tissue.

Regulation of Cell Size Is Dynamic

How the relationship between cell size and cell-cycle progression is orchestrated is less clear. The simplest possibility is that some dimension of the cell is directly limiting, such that division is inhibited until an absolute threshold size is reached (Figure 1B). For example, there may be an absolute lower size limit for mitotic spindle assembly [1]. However, like all other organisms studied, plant cells divide at a range of different sizes according to environmental conditions [18], suggesting that they do not normally divide at this absolute minimum size. A flexible size for division can be created if, instead of directly blocking cell-cycle progression, cell size indirectly inhibits cell-cycle progression via an effect on a process or molecule required for cell division. Because the relationship between this intermediate or proxy and cell size is likely to depend on multiple parameters, division can be triggered at a range of different sizes depending on a combination of internal and external conditions. Importantly, this means that cell size at division is a dynamic process that is determined on a cell-by-cell basis.

Various proxies for cell size have been suggested, including cellular growth rate [24], metabolic rate [25], and the volumetric or geometric ratios between different cellular compartments [26,27]. At the molecular level, information about these processes or dimensions must be integrated into the cell cycle either through cell-size dependent production, activation, or dilution of cell-cycle regulators [28] (Box 2). In rod-shaped fission yeast, the subcellular localisation of upstream regulators of the

Glossary

Absolute growth rate (AGR): the total amount of biomass produced by a cell or tissue per unit time.
Floral primordia: the group of cells that will develop to become a flower.
Meristematic tissues: plant tissues containing undifferentiated and mitotically active cells that support continuous development.
Relative growth rate (RGR): the amount of new growth produced per unit time relative to the starting size of the cell or tissue.
Shoot apical meristem (SAM): a small, domed structure found at the tip of the growing shoots that houses stems cells at its centre and initiates primordia on its flanks that develop into leaves and flowers.

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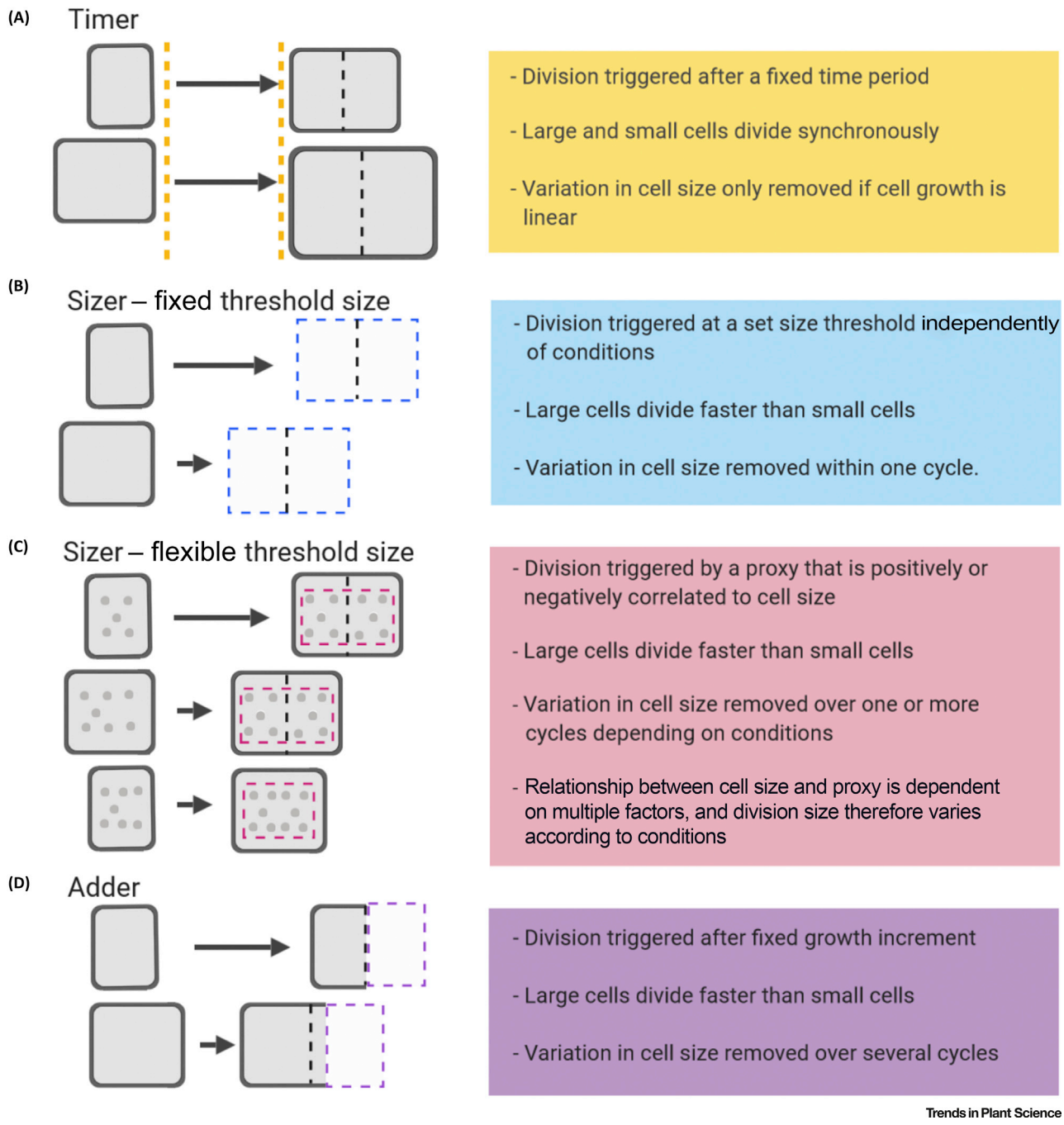

Figure 1. Comparison of Proposed Mechanisms for Cell Size Control.

Multiple theories of cell size control have been proposed [8,9].

The key features of these mechanisms are illustrated above.

(A) In the 'timer' mechanism, there is no active control of cell size. Cells divide after a set amount of time has elapsed. This can result in a steady-state if cells double in size before dividing, but if cells grow exponentially then differences in cell size at the beginning of the cycle will not be removed.

(B) Cell division could be inhibited until a fixed threshold size is reached, for example, the minimum volume required for spindle assembly. If size directly triggers division, variation in cell size would be removed in a single cycle.

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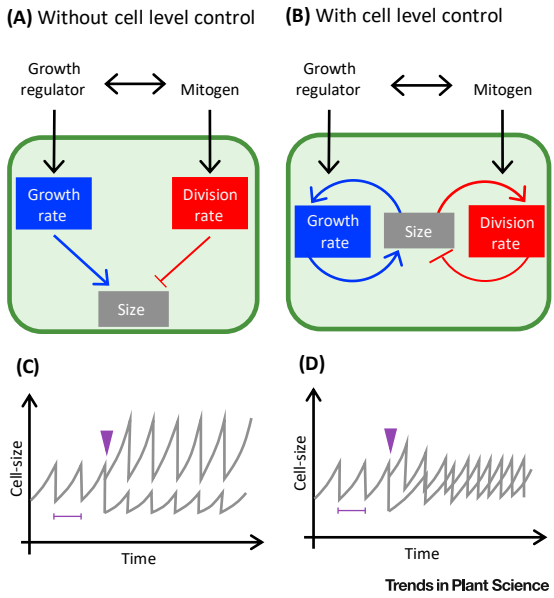

Figure 2. Comparison of Cell Size Control through Tissue- and Cell-Level Mechanisms.
(A) In the first model, cell growth and division are regulated by tissue-level hormonal signals. The ratio of signals received by a cell determines the balance between growth and division, and hence its size at division. Cells with different birth sizes, but receiving the same tissue-level signals, are expected to divide after the same amount of time.
(B) In the second model, cell growth rate and division rate are hypothesized to be dependent on cell size as well as being regulated by tissue-level signals. This creates feedback and coordination between the two processes at the level of the cell. Cells of different birth sizes are expected to divide after different amounts of time despite receiving the same tissue-level signals.
(C) Output of a tissue-level mechanism. Cell size is shown over multiple cycles. The purple bar indicates a single cycle. If growth and division are correctly balanced, cell size will be maintained over successive division cycles. Because growth and division rates are set by tissue-level signals that are received by all cells, large and small cells divide after the same amount of time, and differences in cell size introduced through an unequal division (arrowhead) are not removed in subsequent cycles. Repeated unequal divisions will result in increasing variation in cell size.
(D) In a system including cell-level control, cell-cycle length is inversely dependent on cell size such that large cells divide more rapidly than small cells. Size discrepancies introduced through unequal division (arrowhead) are removed in subsequent cycles.

cell cycle is important in relaying cell size [5,27], but in budding yeast attention has largely focused on how the nuclear concentrations of key cell-cycle regulators themselves change [29–31]. Positive regulators of the plant cell cycle such as the D-type CYCLINS and negative regulators such the KIP-RELATED PROTEINs (KRPs) have antagonistic dose-dependent effects on meristematic cell size [15,18]. Although this appears consistent with a budding yeast-like system, the exact molecular mechanism in plants remains to be determined.

Understanding the exact nature of the molecular system will be important because proxy mechanisms have been shown to produce a range of different outputs based on their structure and dynamics. An

Figure 1. *Continued*
(C) More likely, cells use a proxy to integrate information about cell size into cell-cycle progression. In this example, size-dependent accumulation of a positive regulator of the cell cycle triggers cell division and results in faster cell division in larger cells. Depending on the exact relationship between the trigger and cell size, it may take one or more cycles to remove variation in cell size. The absolute size of cells at division is flexible; for example, if production of the regulator per unit volume of cytoplasm is increased, then cells divide at smaller sizes.
(D) In the final model, cells divide after they have grown or 'added' a fixed increment of volume. If cells divide symmetrically, variation in cell size will be removed over several cycles until the increment added per cycle is equal to the birth size of new cells.

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Box 1. Using Single-Cell Data to Understand Tissue Behaviours

Even within clonal populations, the cell cycle will tend to become asynchronous within a few generations. This makes it difficult to study regulatory changes that take place at specific points in the cell cycle. In unicellular organisms, this problem can be overcome by using culture methods to create synchronised growth and division either by employing a temporary block to the cell cycle or by selecting subsets of cells from mixed populations [53]. Once cell-to-cell variation has been removed, the behaviour of individual cells can easily be inferred from behaviours detected at the population level. For example, experiments where synchronised cultures were split in two, with half in nutrient-rich medium and half in nutrient-poor medium, identified corrective division behaviours that allowed the populations to adapt to their new conditions [54]. These techniques provide large amounts of biological material that allow the detection of proteins and RNAs through blotting techniques and biochemical assays, but may also create abnormal behaviours.

Although arabidopsis cells can be grown in liquid culture and can be synchronised using similar techniques [55], the relevance of the results to developmental contexts is difficult to interpret. Recent advances in live cell imaging and image analysis mean that intact tissues can now be imaged over multiple days and each individual cell and its daughters can be tracked over time as they progress through the cell cycle [46,56–59]. Instead of synchronising the behaviour of cells, cell-to-cell variation is utilised to identify different behaviours. By building computer models based on real measured parameters, the effect of hypothetical growth and division rules can be compared against real tissues [20,23,60,61]. The power of collecting single-cell data can be appreciated by the fact that many studies using unicellular organisms are now also collecting data at the single-cell level [29,62]. In contrast to synchronisation methods, time-lapse methods do not produce large amounts of material for biochemical analysis, and therefore advances in the development of genetically encoded sensors for cyclin-dependent kinase (CDK) activity and single-cell omic technologies will be necessary to complement this new level of detail.

interesting phenomenon that has recently been described is that proxy sizer mechanisms can behave as 'adders' – meaning that all cells add the same absolute volume per cycle (Figure 1D) [31,32]. Detailed statistical analysis demonstrated SAM cells do not behave as classic sizers, but neither do they match the behaviour expected of classic adders [16]. New models will therefore be necessary to understand how the intermediate behaviours shown by SAM cells could be generated. Detailed quantitative data, particularly regarding the production, degradation, and dilution of cell cycle regulators, will be necessary to inform these models. This raises significant technical challenges because cell-cycle regulators are often expressed at very low levels and are highly labile, but recent studies have successfully detected and characterised stochastic variation in the expression of gene products between neighbouring cells [33,34], and similar approaches may also be successful here. It may also be necessary to consider the potential effects of multiple mechanisms acting at different stages of the cell cycle [31,32], the effect of cell size on growth rate [16,24,35], and the potential for additional triggers of division such as tensile stress [36] or local topological networks [21,23] that could override cell size control.

Dynamic Cell Size Control as a Tool in Multicellular Development

To fully understand cell size control and its effects in a multicellular context, multilevel models that accurately predict both tissue-level and cell-level behaviours will be required. Historically, many computer models of plant tissue growth have used a perfect sizer rule to trigger cell division as soon as a fixed threshold size is reached [22,37–39]. We believe that models that can more accurately predict changes in cell size may improve our understanding of the principles of multicellular development.

First, the relationship between **relative growth rate (RGR)** and cell size might play a role in establishing and maintaining basic tissue structures. RGR is a strong predictor of cell size at division in size-dependent models of cell-cycle progression [8], and differences in RGR across a tissue can therefore lead to gradients of cell sizes and contribute to establishing complex tissue structures such as those found in the leaf epidermis [40]. Local differences in RGR might be the result of mechanical or physiological constraints imposed by tissue structure itself, or the result of developmental or environmental signals that reprogramme cellular metabolism or cell-wall structure. Developmentally imposed metabolic restrictions are common in mammalian tissues where cell growth is primarily driven by an increase in cytoplasmic volume.

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Box 2. Searching for Sizers in the Plant Cell Cycle

The eukaryotic cell cycle depends on CYCLIN-DEPENDENT KINASE (CDK) activity reaching critical levels to activate stage-specific transcriptional regulators [63,64]: the first transition initiates DNA synthesis (G1/S), and the second triggers partitioning of the nuclear and cell contents through the process of mitosis (G2/M). CDK activity is regulated by interacting proteins that could integrate information about metabolic status and cell size into cell-cycle progression (Figure I). Size-dependent cell-cycle regulation could be achieved either through accumulation of an activator or by the dilution of an inhibitor [8].

CYCLIN (CYC) proteins are the major activating subunits of CDK complexes and are either highly labile or degraded at the end of each cycle [65]. The synthesis of CYC proteins is tightly linked to the metabolic capacity of the cell [66], and a larger cytoplasmic volume with associated higher biosynthetic capacity is therefore predicted to attain required threshold level of nuclear CDK activity more rapidly than a smaller cell. Because nuclear volume scales with cell size through the cell cycle [16,67], a mechanism for counting active CDK molecules within the nucleus is required. This could be achieved by titrating active transcription factors that are the downstream effectors of CDK signalling against the fixed number of binding sites in the genome [8,30]. This hypothesis explains the well-documented relationship between the number of genome copies and cell size [67–69], but there is only limited supporting experimental evidence [30].

In addition to D-type and B-type CYCs, which in plants are rate-limiting for the G1/S and G2/M transitions, respectively [70,71], phase-specific accumulation of transcriptional activators such as E2F [72] at G1/S and three-repeat MYBs at G2/M [73] could also contribute to initiator/accumulator-type mechanisms. The plant-specific mitotic CDKBs [74], which unlike CDKA accumulate during the cell cycle, offer an additional point of control.

Negative regulators of the cell cycle can also provide cell size control if diluted or degraded as the cell grows [8]. The best evidence for this comes from yeast, where dilution of the transcriptional repressor Whi5 is necessary for G1/S [29]. In plants the RETINOBLASTOMA-RELATED (RBR) protein negatively regulates S-phase genes and could play a similar role [75], as could negatively acting three-repeat MYB transcription factors that inhibit G2/M [76]. Plants also possess CDK inhibitors, such as the KIP-RELATED PROTEINS, which inhibit CDK activity at both the G1/S and G2/M transitions [77] and are known to affect cell size. Quantification of plant cell-cycle regulators together with further modelling will identify which of the potential models for cell size regulation are feasible.

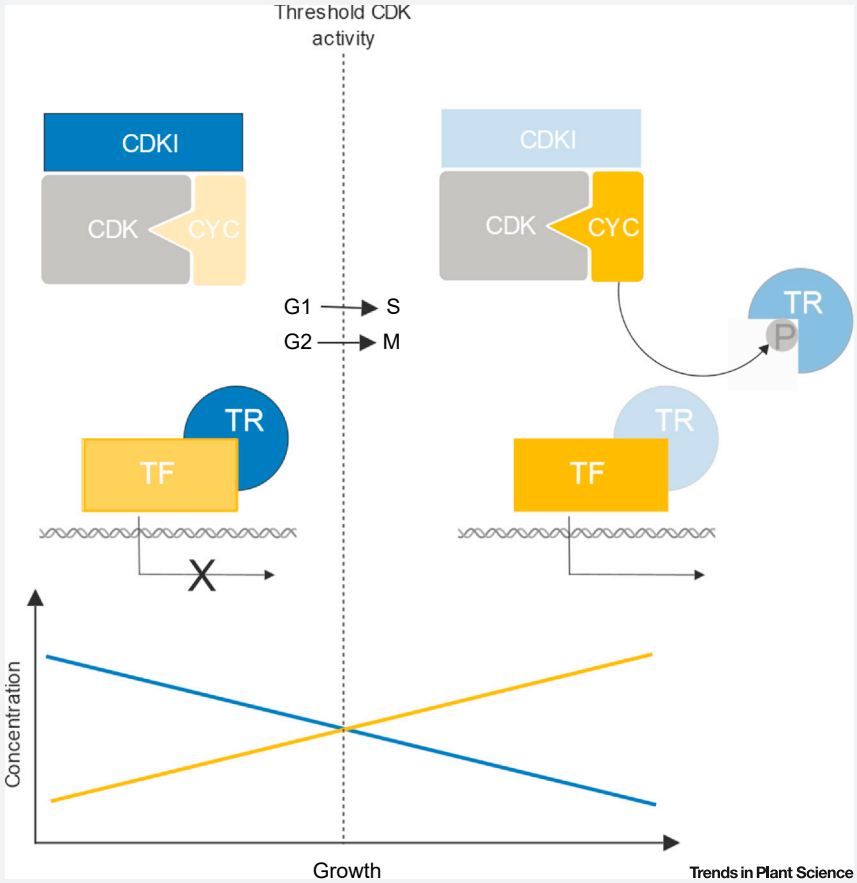

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Figure I. Generalised Model for Cell Size-Dependent Cell-Cycle Progression

Schematic showing a generalised cell-cycle regulatory module based on increasing CYCLIN-DEPENDENT KINASE (CDK) activity. The concentration of CDK (grey) remains constant, but interacting proteins and downstream targets with activating (yellow) or inhibitory (blue) effects on cell-cycle progression change in concentration as the cell grows. Before reaching the CDK activity threshold, the expression of genes regulated by a phase-specific activating transcription factor (TF) is blocked by the presence of a transcriptional repressor (TR). Phosphorylation (P) of the TR by CDK is necessary to relieve transcriptional repression and initiate phase-specific activities. A size-dependent increase in CDK activity could be achieved if either the production of CYCLIN (CYC) activators increases as the cell grows, as a result of increased biosynthetic capacity, or if the concentration of CDK inhibitor protein (CDKI) is diluted as the cell increases in volume. The amount of CDK activity required for transition could also be lowered as cell size increases through increased production of positive-acting TFs or dilution of negative-acting TRs.

Loss of these mechanisms in cancer cells results in 'greedy' behaviour that allows them to grow and proliferate more quickly than their healthy neighbours [41]. In plants, attention has historically focused mainly on turgor-driven growth, but more recently the role of cytoplasmic growth has also been highlighted. For example, the TARGET OF RAPAMYCIN pathway, which regulates the basal protein synthesis rate by controlling the number of active ribosomes, and plays important roles in regulating cell size in response to sugar and nitrate signalling in yeast and animal cells, has also been identified in plants [42].

Second, cell size will affect the **absolute growth rate (AGR)** of the tissue because a tissue made up of large cells will grow at a higher AGR than a tissue composed of the same number of smaller cells with the same RGR (Figure 3A). Many domesticated species such as potato accumulate more biomass than their wild relatives as the result of a global increase in cell size related to genome duplication [43,44], but local changes in cell size might also be used developmentally to increase AGR in specific tissues. Because increased RGR is itself predicted to shift the system towards larger cell sizes, an amplifying effect may be created that could be a useful morphogenic tool (Figure 3B,C). During the initiation of floral organs, where auxin locally increases RGR, a concurrent increase in cell size is also observed [18,45,46]. It would be interesting to know whether this increase in cell size contributes to the rate of biomass accumulation.

Third, cell size is likely to affect signalling. Positional information is crucial during plant development and is often mediated by mobile signals that form instructive gradients or feedback loops between cells [47]. The distribution of signalling molecules is therefore important, as is the structure of the tissue that will receive and respond to the signal. This effect has been demonstrated by comparing patterning in **floral primordia** with the same overall tissue area but in cells of a different size [15]. When cells were larger, and consequently fewer, sepal primordia could not be positioned correctly, and neighbouring organs were often fused. Cell size might also affect the distribution of passively and actively transported signalling molecules. This is particularly interesting where the signalling molecules might themselves affect cell size, creating feedback [38,48–51]. Understanding the role of cell size in feedbacks such as these will be central to answering the question of whether developmental domains are defined in terms of absolute dimensions or cell numbers.

Finally, cell size regulation may affect synchronicity within the tissue. This is because the more size corrections are made through longer or shorter cycles, the more asynchronous the cell cycle becomes between neighbours (Figure 2). In arabidopsis (*Arabidopsis thaliana*) root, most divisions are symmetric, and groups of up to eight clonally related cells initiate DNA synthesis synchronously [52], but in the SAM, where uneven divisions are more frequent and size corrections are required, the onset of DNA synthesis is less synchronised [15,18]. The potential significance of cell-cycle position for morphogenesis is illustrated by the recent finding that that only cells in the G2 phase of the cell cycle are competent to respond to a signal that specifies the specialised 'giant' cell type in the sepal epidermis [33]. Cell size-related breaking of synchronicity might therefore be advantageous in ensuring a constant supply of cells for differentiation, and might also contribute to spacing between specialised cell types.

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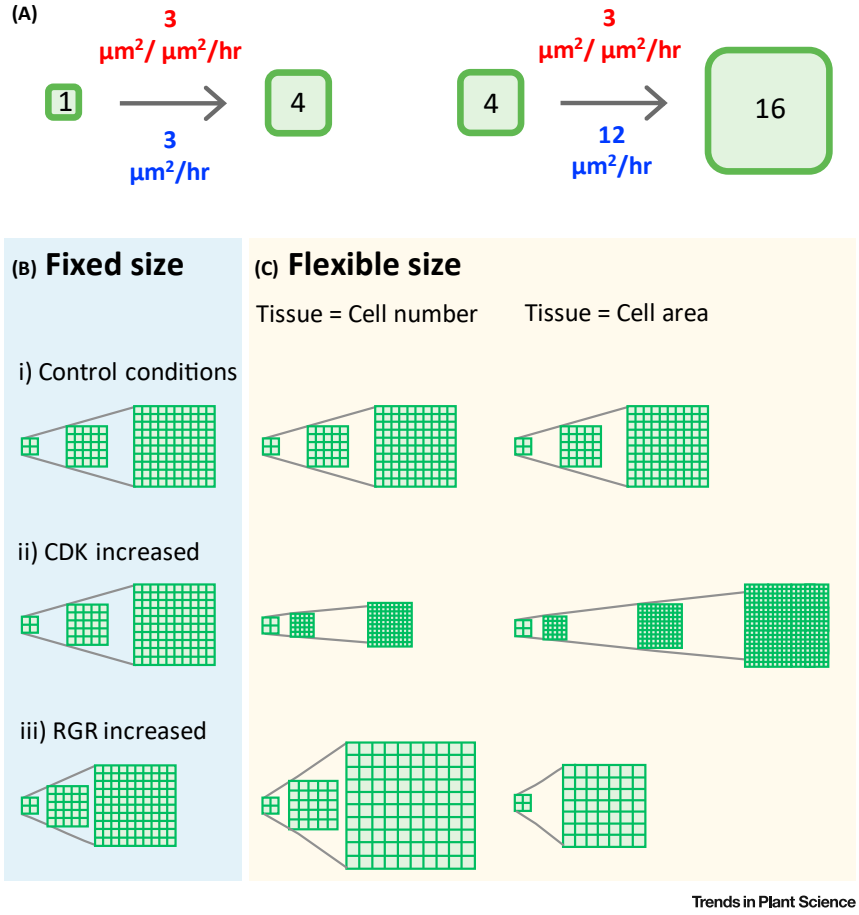

Figure 3. Cell Size Control May Affect Tissue Growth and Development

(A) Comparison of the area (black), relative growth rate (RGR, red), and absolute growth rate (AGR, blue) of two cells with different birth sizes. Both cells grow at the same RGR, but the small cell has a lower AGR and adds less biomass than the large cell in the same time period.

(B) Putative outputs of a tissue-level model in which cell division is triggered when a fixed threshold size is reached. If all cells divide at the same absolute size irrespective of conditions (examples i–iii), the same tissue structure is always produced.

(C) If cell size at division is flexible, the resulting tissue structure will vary according to conditions (examples i–iii). If the final tissue is defined by the total number of cells, conditions that alter cell size affect the total area of the tissue. If the final tissue is defined by its total area, conditions that alter cell size will alter total number of cells. Integration of cell-level models of division into tissue-level models of tissue growth and signalling will allow these relationships to be explored further.

Concluding Remarks and Future Perspectives

Current studies are uncovering increasingly strong links between cell size control in unicellular organisms and meristematic plant tissues. Instead of dividing according to fixed rules imposed by tissue-level signals, plant cells show much of the same flexibility and autonomy displayed by unicellular organisms. New models will now be necessary to understand how these same behaviours, that allow unicellular organisms to adapt to changes in the environment, contribute to the generation of complex cellular structures in multicellular organisms. This will involve improving our understanding of the molecular basis of cell-level models and integrating this knowledge into tissue-level models.

Outstanding Questions

How do the dynamics of the network of cell-cycle regulators contribute to determining cell size?

Are some cell cycles not controlled by cell size?

Do cells driven primarily by cytoplasmic growth control cell size in a different way from cells driven primarily by turgor-driven growth?

Does cell size control change when meristematic cells start to differentiate and become more vacuolated?

Are developmental changes in cell size important in amplifying or fine-tuning absolute tissue growth rates?

What feedback loops exist between cell size, the distribution of morphogens, and the regulation of cell growth, and what does this tell us about whether tissue area is specified in terms of area or cell number?

What does the regulation of cell size in plants tell us about the evolution of multicellularity? To what extent might the existence of ancestral cell size control mechanisms have contributed to multicellular development? How universal are mechanisms controlling growth in multicellular organisms?

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Although the network of regulators that control the cell cycle in plants has been well described, quantitative information regarding the accumulation and dilution regulators will be necessary to understand the dynamics that lead to cell size control at cell-cycle checkpoints. In this work it will be relevant to question whether cell size is controlled by a single mechanism, or if multiple mechanisms are necessary to produce the patterns of cell size observed in developing tissues. It will also be interesting to consider whether accumulation and dilution mechanisms have been actively selected during evolution, or if they are simply a consequence of reactions being contained within cells. Plant cells also offer an opportunity to increase our fundamental understanding of cell size control by studying autotrophic cells that combine cytoplasmic growth with the potential for expansion by turgor-driven growth.

Because plants develop continuously from meristematic tissues, the regulation of cell size in dividing tissues is of particular importance. Understanding these rules, and how they might affect processes at different levels of organisation, will not only allow us to understand better how plants grow and respond to external signals but might also allow us to make predictable changes to tissue structures in the future (See Outstanding Questions).

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References

1. Marshall, W.F. *et al.* (2012) What determines cell size? *BMC Biol.* 10, 101
2. Bassel, G.W. *et al.* (2014) Mechanical constraints imposed by 3D cellular geometry and arrangement modulate growth patterns in the *Arabidopsis* embryo. *Proc. Natl. Acad. Sci. U. S. A.* 111, 8685–8690
3. Hetherington, A.M. and Woodward, F.I. (2003) Environmental change. *Nature* 424, 901–908
4. Lloyd, A.C. (2013) The regulation of cell size. *Cell* 154, 1194–1205
5. Wood, E. and Nurse, P. (2015) Sizing up to divide: mitotic cell-size control in fission yeast. *Annu. Rev. Cell Dev. Biol.* 31, 11–29
6. Tsukaya, H. (2002) Interpretation of mutants in leaf morphology: genetic evidence for a compensatory system in leaf morphogenesis that provides a new link between cell and organismal theories. *Int. Rev. Cytol.* 217, 1–39
7. Horiguchi, G. and Tsukaya, H. (2011) Organ size regulation in plants: insights from compensation. *Front. Plant Sci.* 2, 24
8. Fantes, P.A. *et al.* (1975) The regulation of cell size and the control of mitosis. *J. Theor. Biol.* 50, 213–244
9. Fantes, P. and Nurse, P. (1981) *The Cell Cycle*, Cambridge University Press
10. Jorgensen, P. and Tyers, M. (2004) How cells coordinate growth and division. *Curr. Biol.* 14, 1014–1027
11. Miller, C.O. *et al.* (1955) Kinetin, a cell division factor from deoxyribonucleic acid 1. *J. Am. Chem. Soc.* 77, 1392–1392.
12. Müller, B. and Sheen, J. (2008) Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis. *Nature* 453, 1094–1097
13. Zhao, Z. *et al.* (2010) Hormonal control of the shoot stem-cell niche. *Nature* 465, 1089–1092
14. Uyttewaal, M. *et al.* (2012) Mechanical stress acts via katanin to amplify differences in growth rate between adjacent cells in *Arabidopsis*. *Cell* 149, 439–451
15. Serrano-Mislata, A. *et al.* (2015) Active control of cell size generates spatial detail during plant organogenesis. *Curr. Biol.* 25, 2991–2996
16. Willis, L. *et al.* (2016) Cell size and growth regulation in the *Arabidopsis thaliana* apical stem cell niche. *Proc. Natl. Acad. Sci. U. S. A.* 113, E8238–E8246
17. Kwiatkowska, D. and Dumais, J. (2003) Growth and morphogenesis at the vegetative shoot apex of *Anagallis arvensis* L. *J. Exp. Bot.* 54, 1585–1595
18. Jones, A.R. *et al.* (2017) Cell-size dependent progression of the cell cycle creates homeostasis and flexibility of plant cell size. *Nat. Commun.* 8, 15060
19. Asl, L.K. *et al.* (2011) Model-based analysis of *Arabidopsis* leaf epidermal cells reveals distinct division and expansion patterns. *Plant Physiol.* 156, 2172–2183
20. Elsner, J. *et al.* (2012) Spatiotemporal variation of leaf epidermal cell growth : a quantitative analysis of *Arabidopsis thaliana* wild-type and triple cyclinD3 mutant plants. *Ann. Bot.* 109, 897–910
21. Gibson, W.T. *et al.* (2011) Theory control of the mitotic cleavage plane by local epithelial topology. *Cell* 144, 427–438
22. Shapiro, B.E. *et al.* (2015) Analysis of cell division patterns in the *Arabidopsis* shoot apical meristem. *Proc. Natl. Acad. Sci. U. S. A.* 112, 2–7
23. Jackson, M.D.B. *et al.* (2019) Global topological order emerges through local mechanical control of cell divisions in the *Arabidopsis* shoot apical meristem. *Cell Syst.* 8, 53–65
24. Björklund, M. (2019) Cell size homeostasis: metabolic control of growth and cell division. *BBA - Mol. Cell Res.* 1866, 409–417
25. Siqueira, J.A. *et al.* (2018) Unraveling interfaces between energy metabolism and cell cycle in plants. *Trends Plant Sci.* 23, 731–747
26. Gregory, T.R. (2001) Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biol. Rev. Camb. Philos. Soc.* 76, 65–101
27. Facchetti, G. *et al.* (2019) Reprogramming Cdr2-dependent geometry-based cell size control in fission yeast. *Curr. Biol.* 29, 350–358
28. Schmoller, K.M. and Skotheim, J.M. (2015) The biosynthetic basis of cell size control. *Trends Cell Biol.* 25, 793–802
29. Schmoller, K. *et al.* (2015) Dilution of the cell cycle inhibitor Whi5 controls budding yeast cell size. *Nature* 526, 268–272
30. Wang, H. *et al.* (2009) Recruitment of Cln3 cyclin to promoters controls cell cycle entry via histone

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deacetylase and other targets. *PLoS Biol.* 7, e1000189
31. Heldt, F.S. *et al.* (2018) Dilution and titration of cell-cycle regulators may control cell size in budding yeast. *PLoS Comput. Biol.* 14, 1–26
32. Chandler-Brown, D. *et al.* (2017) The adder phenomenon emerges from independent control of pre- and post-start phases of the budding yeast cell cycle. *Curr. Biol.* 27, 2774–2783
33. Meyer, H.M. *et al.* (2017) Fluctuations of the transcription factor atml1 generate the pattern of giant cells in the *Arabidopsis* sepal. *Elife* 6, e19131
34. Ietswaart, R. *et al.* (2017) Cell-size-dependent transcription of FLC and its antisense long non-coding RNA COOLAIR explain cell-to-cell expression variation. *Cell Syst.* 4, 622–635
35. Cadart, C. *et al.* (2018) Size control in mammalian cells involves modulation of both growth rate and cell cycle duration. *Nat. Commun.* 9, 3275
36. Louveaux, M. *et al.* (2016) Cell division plane orientation based on tensile stress in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U.S.A.* 113, E4294–E4303
37. Sahlin, P. and Jönsson, H. (2010) A modeling study on how cell division affects properties of epithelial tissues under isotropic growth. *PLoS One* 5, e11750
38. Smith, R.S. *et al.* (2006) A plausible model of phyllotaxis. *Proc. Natl. Acad. Sci. U. S. A.* 103, 1301–1306
39. Jönsson, H. *et al.* (2006) An auxin-driven polarized transport model for phyllotaxis. *Proc. Natl. Acad. Sci. U. S. A.* 103, 1633–1638
40. Fox, S. *et al.* (2018) Spatiotemporal coordination of cell division and growth during organ morphogenesis. *PLoS Biol.* 16, e2005952
41. Wu, W. and Zhao, S. (2013) Metabolic changes in cancer: beyond the Warburg effect. *Acta Biochim. Biophys. Sin. (Shanghai)* 45, 18–26
42. Xiong, Y. *et al.* (2013) Glucose–TOR signalling reprograms the transcriptome and activates meristems. *Nature* 496, 181
43. Salman-Minkov, A. *et al.* (2016) Whole-genome duplication as a key factor in crop domestication. *Nat. Plants* 2, 1–4
44. Meyer, R.S. *et al.* (2012) Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New Phytol.* 196, 29–48
45. Reinhardt, D. *et al.* (2000) Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* 12, 507–518
46. Fernandez, R. *et al.* (2010) Imaging plant growth in 4D: robust tissue reconstruction and lineaging at cell resolution. *Nat. Methods* 7, 547–553
47. Pallakies, H. and Simon, R. (2010) Positional information in plant development. *Encycl. Life Sci.* Published online April 189, 2010. https://doi.org/10.1002/9780470015902.a0002069.pub2
48. Band, L.R. *et al.* (2014) Systems analysis of auxin transport in the *Arabidopsis* root apex. *Plant Cell* 26, 862–875
49. Grieneisen, V.A. *et al.* (2007) Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* 449, 1008–1013
50. Gordon, S.P. *et al.* (2009) Multiple feedback loops through cytokinin signaling control stem cell number within the *Arabidopsis* shoot meristem. *Proc. Natl. Acad. Sci. U. S. A.* 106, 16529–16534
51. Gruel, J. *et al.* (2016) An epidermis-driven mechanism positions and scales stem cell niches in plants. *Sci. Adv.* 2, e1500989
52. Kumpf, R. *et al.* (2014) The ASH1-RELATED3 SET-domain protein controls cell division competence of the meristem and the quiescent center of the *Arabidopsis* primary root. *Plant Physiol.* 166, 632–643
53. Walker, G.M. (1999) Synchronization of yeast cell populations. *Methods Cell Sci.* 21, 87–93
54. Fantes, P. and Nurse, P. (1977) Control of cell size at division in fission yeast by a growth-modulated size control over nuclear division. *Exp. Cell Res.* 107, 377–386
55. Menges, M. and Murray, J.A.H. (2002) Synchronous *Arabidopsis* suspension cultures for analysis of cell-cycle gene activity. *Plant J.* 30, 203–212
56. Barbier de Reuille, P. *et al.* (2015) MorphoGraphX: a platform for quantifying morphogenesis in 4D. *Elife* 4, 1–20
57. von Wangenheim, D. *et al.* (2017) Live tracking of moving samples in confocal microscopy for vertically grown roots. *Elife* 6, e26792
58. Montenegro-Johnson, T. *et al.* (2019) 3DCellAtlas Meristem – a tool for the global cellular annotation of shoot apical meristems. *Plant Methods* 15, 33
59. Rahni, R. and Birnbaum, K.D. (2019) Week-long imaging of cell divisions in the *Arabidopsis* root meristem. *Plant Methods* 15, 30
60. Roeder, A.H.K. *et al.* (2010) Variability in the control of cell division underlies sepal epidermal patterning in *Arabidopsis thaliana*. *PLoS Biol* 8, e1000367
61. Roeder, A.H.K. *et al.* (2012) Cell cycle regulates cell type in the *Arabidopsis* sepal. *Development* 139, 4416–4427
62. Tanouchi, Y. *et al.* (2015) A noisy linear map underlies oscillations in cell size and gene expression in bacteria. *Nature* 523, 357–359
63. Coudreuse, D. and Nurse, P. (2010) Driving the cell cycle with a minimal CDK control network. *Nature* 468, 1074–1079
64. Gérard, C. *et al.* (2015) Cell cycle control by a minimal Cdk network. *PLoS Comput. Biol.* 11, e1004056
65. Evans, T. *et al.* (1983) Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* 33, 389–396
66. Polymenis, M. and Schmidt, E.V. (1997) Coupling of cell division to cell growth by translational control of the G1 cyclin CLN3 in yeast. *Genes Dev.* 11, 2522–2531
67. Robinson, D.O. *et al.* (2018) Ploidy and size at multiple scales in the *Arabidopsis* sepal. *Plant Cell* 30, 2308–2329
68. Li, X. *et al.* (2012) Developmental, cytological and transcriptional analysis of autotetraploid *Arabidopsis*. *Planta* 236, 579–596
69. Del Pozo, J.C. and Ramirez-Parra, E. (2015) Whole genome duplications in plants: an overview from *Arabidopsis*. *J. Exp. Bot.* 66, 6991–7003
70. Menges, M. *et al.* (2006) The D-type cyclin CYCD3;1 is limiting for the G1-to-S-phase transition in *Arabidopsis*. *Plant Cell* 18, 893–906
71. Schnittger, A. *et al.* (2005) Ectopic B-type cyclin expression induces mitotic cycles in endoreduplicating *Arabidopsis* trichomes. *Curr. Biol.* 15, 415–420
72. Menges, M. *et al.* (2005) Global analysis of the core cell cycle regulators of *Arabidopsis* identifies novel genes, reveals multiple and highly specific profiles of expression and provides a coherent model for plant cell cycle control. *Plant J.* 41, 546–566
73. Haga, N. *et al.* (2007) R1R2R3-Myb proteins positively regulate cytokinesis through activation of KNOLLE transcription in *Arabidopsis thaliana*. *Development* 134, 1101–1110
74. Segers, G. *et al.* (1996) The *Arabidopsis* cyclin-dependent kinase gene cdc2bat is preferentially expressed during S and G2 phases of the cell cycle. *Plant J.* 10, 601–612

75. Uemukai, K. *et al.* (2005) Transcriptional activation of tobacco E2F is repressed by co-transfection with the retinoblastoma-related protein: cyclin D expression overcomes this repressor activity. *Plant Mol. Biol.* 57, 83–100

76. Chen, P. *et al.* (2017) *Arabidopsis* R1R2R3-Myb proteins are essential for inhibiting cell division in response to DNA damage. *Nat. Commun.* 8, 635

77. Veylder, L. De *et al.* (2007) Functional analysis of cyclin-dependent kinase inhibitors of *Arabidopsis*. *Plant Cell* 13, 1653