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Cell cycle and differentiation

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The development of multicellular organisms relies on the temporal and spatial control of cell proliferation and cell growth. The relationship between cell-cycle progression and development is complex and characterized by mutual dependencies. On the level of the individual cell, this interrelationship has implications for pattern formation and cell morphogenesis. On a supercellular level, this interrelationship affects meristem function and organ growth. Often, developmental signals not only direct cell-cycle progression but also set the frame for cell-cycle regulation by determining cell-type-specific cell-cycle modes. In other cases, however, cell-cycle progression appears to be required for the further differentiation of some cell types. There are also examples in which cell cycle and differentiation seem to be controlled at the same level and progress rather independently from each other or are linked by the same regulator or pathway. Furthermore, different relationships between cell cycle and differentiation can be combined in a succession of events during development, leading to complex developmental programs.

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Abbreviations

APC/C	anaphase-promoting complex/cyclosome
C	DNA content corresponding to haploid set of chromosomes
CCS52	CELL-CYCLE SWITCH 52
CDC6	CELL DIVISION CYCLE DEFECTIVE 6
CDK	cyclin-dependent kinase
CDT1	*cdc10*-DEPENDENT TRANSCRIPT1
CUC	*CUP-SHAPED COTELYDON*
CYC	CYCLIN
dn	dominant-negative
DPa	*DIMERIZATION PARTNERa*
E2F	adenovirus E2 promoter binding factor
FZR	FIZZY-RELATED
GFP	green fluorescent protein
ICK1	*INTERACTOR/INHIBITOR OF CDKs1*
KRP2	*KIP-RELATED PROTEIN2*

Introduction

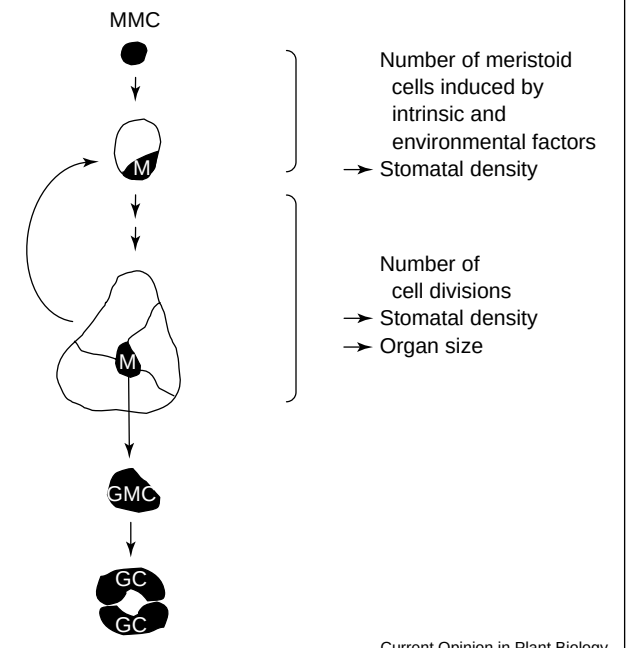
The study of cell-cycle control in plants presents two major opportunities. First, one can learn about the general cell-cycle machinery. This has the potential not only to reveal new, plant-specific control mechanisms but also to contribute to the understanding of canonical cell-cycle regulation and to provide insights relating to the evolution of cell-cycle regulation. This review, however, deals with the second opportunity arising from cell-cycle analyses in plants, that is, the chance to study cell-cycle progression in the developmental context of a multicellular eukaryote.

Throughout plant life-cycle, intensive crosstalk between cell-cycle control and differentiation processes is required for proper development. This interrelationship functions at different levels. On the level of the individual cell, cell-cycle progression has to be coordinated with pattern formation, especially as cells are often specified in a domain of rapid cell divisions. After a certain fate has been adopted, cell cycle has to be dove-tailed with the regulation of cell morphogenesis and physiology. Finally, cell-division and cell-enlargement patterns have to be controlled on a supercellular level (that of the tissue or organ). Supercellular control is essential, for example, to maintain meristem homeostasis and organ growth and to respond to an ever-changing environment. Here, we review recent results obtained from the study of single cells as well as from studies of tissue or organ development. We try to deconstruct and categorize these findings into different classes of interactions between cell cycle and differentiation.

Cell cycle and cell-fate specification

A very instructive example of how cell division is interrelated with cell differentiation is the development of stomata in *Arabidopsis* (Figure 1; for a detailed description of stomatal development see recent reviews [1,2]). First, a meristemoid mother cell divides unequally to give rise to a larger cell, which will develop into an epidermal pavement cell, and a smaller cytologically distinct meristemoid cell. The meristemoid is comparable to an animal transit amplifying cell, that is, a cell with limited stem-cell character. It may divide asymmetrically several times, each time producing a new epidermal cell and regenerating the meristemoid. After a few rounds of division, meristemoids withdraw from the cell-division cycle and terminally differentiate into a guard mother cell, which divides symmetrically to generate two guard cells comprising the stoma. Although most other cells that are produced by this lineage eventually differentiate as pavement cells, some reinitiate a

Figure 1



Stomatal development. **(a)** On the cellular level, a single cell differentiates into a meristemoid mother cell (MMC), the MMC then divides unequally to give rise to a larger cell, which will develop into an epidermal pavement cell, and a smaller meristemoid cell (M). The meristemoid may divide asymmetrically several times, each time producing a new epidermal cell and regenerating the meristemoid. After a few rounds of division, meristemoids withdraw from the cell-division cycle and terminally differentiate into a guard mother cell (GMC) that divides symmetrically to generate two guard cells (GC) that comprise the stoma. Although most of the other cells produced by this lineage eventually differentiate as pavement cells, some reinitiate a stomatal lineage by dividing asymmetrically to give rise to satellite meristemoids. **(b)** On a supercellular level, the numbers of meristemoid mother and satellite meristemoid cells are controlled by developmental (intrinsic) as well as environmental (extrinsic) factors to achieve an appropriate stomata density. As cell-division of meristemoids produces the majority of epidermal cells, the number of these cell divisions is also under the control of organ size. Modified after del Mar Castellano *et al*. [4•].

stomatal lineage by dividing asymmetrically to give rise to satellite meristemoids.

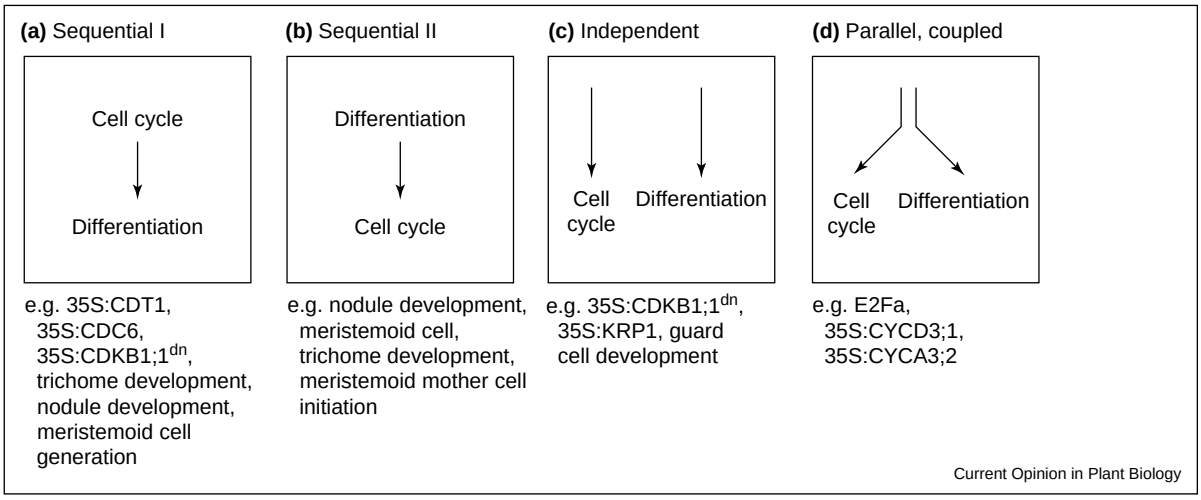
Key to the patterning of stomata is the recruitment of meristemoid mother cells and satellite meristemoids, which has been speculated to involve mechanisms similar to those that regulate the formation of stem cells in the shoot apical meristems [3]. In addition, cell-cycle regulation appears to influence the formation of meristemoids, as was reported by del Mar Castellano and co-workers [4•] who studied the function of CELL DIVISION CYCLE DEFECTIVE6 (CDC6) and *cdc10*-DEPENDENT TRANSCRIPT1 (CDT1), two components that regulate the licensing of origins of replication required for subsequent DNA replication. Plants that misexpressed *CDT1* or *CDC6* from the *35S* promoter showed a two-fold increase in the density of stomata on their leaves, indicating a higher generation of satellite meristemoids and/or meristemoid mother cells that eventually develop into stomata. This suggests that the competence to develop into a transit amplifying meristemoid cell might be controlled directly by the potential to replicate and/or to proceed into a G_2-like state; this is an example in which cell-cycle decisions appear to precede differentiation (Figure 2a).

Another link between cell-division control and the formation of meristemoid cells comes from the work of Boudolf and colleagues [5••]. Their work showed that a B-type cyclin-dependent kinase gene, *CDKB1;1*, is specifically expressed and required for the proper cell-division pattern in the stomatal lineage. The misexpression of a dominant-negative (dn) CDKB1;1 mutant protein, CDKB1;1^{dn}, resulted in reduced leaf size and fewer but larger leaf cells. Strikingly, stomatal complexes were significantly underrepresented in comparison to the total number of leaf cells, indicating that the initiation and/or maintenance of a stem-cell-like activity may depend on a temporally and spatially correct execution of a cell-division program (Figure 2a). One possibility could be that the initial asymmetric division is inherently connected with adoption of stem-cell identity. This connection might involve, for example, resetting cell fate through the execution of mitosis, thus, allowing a new fate to be adopted. A G_2 phase might be of special importance in the preparation for this unequal cell division.

Support for the importance of a G_2 phase for cell-fate specification in plants comes from studies of CYCB2;2. Expression of the alfalfa *CYCB2;2* gene was found to strongly promote entry into mitosis in tobacco BY2 cells, and root generation from leaf discs failed in transgenic tobacco plants that misexpressed *CYCB2;2* [6•]. Determination of the DNA content of leaf-disc cells confirmed that the proportion of cells in a G_2 phase in the *CYCB2;2* misexpression line was strongly reduced in comparison to wildtype [6•].

A G_2 phase and a subsequent proper execution of a cell-division program also appear to be crucial for the initiation of lateral roots, a process that has some similarities to the initial unequal division in the stomatal lineage. For the generation of lateral roots, pericycle cells have to reenter a cell-division cycle that generates founder cells. It has been suggested that pericycle cells reside in a G_2 state before lateral root initiation [7,8], and auxin is probably involved in establishing this state [9]. This finding has been substantiated by recent transcript-profiling experiments on initiating lateral roots, which made use of the observation that plants treated with the auxin transport

Figure 2



Synopsis of different relationships between cell-cycle progression and differentiation. **(a)** Cell-cycle progression determines further differentiation; for example, more meristemoid cells are formed in *35S:CDT1* plants than in wildtype plants. **(b)** Developmental signals control further cell-cycle progression; for example, trichomes start to endoreplicate after being specified. **(c)** Cell cycle and differentiation can progress independently of each other; for example, guard cell differentiation commences without a formative division of a guard mother cell. **(d)** Cell cycle and differentiation can be controlled by the same regulator or pathway; for example, the transcription factor E2F controls the expression of both cell-cycle genes and genes that are involved in differentiation. During development, a succession of different relationships between cell cycle and differentiation is often found. For example, during the formation of stomata, some cells are specified as meristemoid mother cells by developmental signals, which also determine a cell-type-specific cell-cycle program (b). Next, meristemoid mother cells apparently have to go through a cell division to create a meristemoid cell (a), which differentiates eventually into a guard mother cell. Finally, the development of a guard mother cell into two guard cells appears to be largely independent of further cell divisions (c).

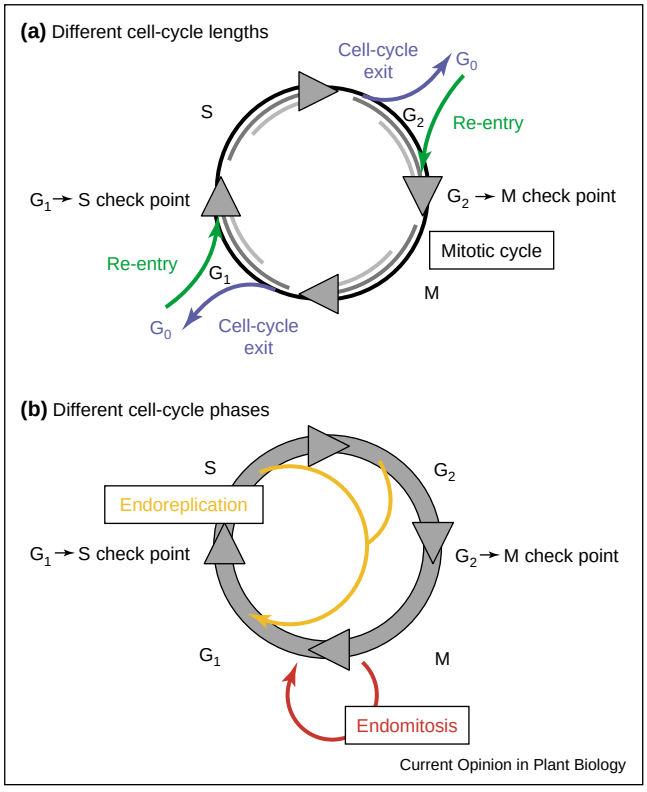
inhibitor nitropropionic acid (NPA) developed no lateral roots. After application of auxin, the first cell-cycle genes found to be upregulated were $G_1 \rightarrow S$ regulators, followed by $G_2 \rightarrow M$ regulators, intriguingly, CDKB1;1 was among those [10•]. And indeed, CDKB1;1^{dn} plants have a reduced number of lateral roots (I De Smet, T Beeckman, pers. comm.).

The number of lateral roots was also found to be reduced in plants that misexpressed the CDK inhibitor *KINASE INHIBITORY PROTEIN* (*KIP*)*-RELATED PROTEIN2* (*KRP2*) [9]. *KRP2*-misexpressing plants also displayed a strong leaf phenotype, which was superficially very similar to that of *CDKB1;1^{dn}* lines, comprising fewer and larger cells but remarkably no significant change in stomatal density. These observations demonstrate that cell-cycle regulators can have different functions depending on the cell type [11].

Interestingly, once a guard mother cell is formed, the blocking of cell division appears not to completely attenuate cell differentiation because kidney-shaped cells resembling one half of a stoma were found in *35S:CDKB1;1^{dn}* plants [5••]. Conversely, exit from a mitotic cycle is not a prerequisite for guard cell differentiation, as shown by the *four lips* mutant in which stomatal complexes with multiple guard cells are formed by cell division from one guard mother cell ([12]; JA Nadeau, FD Sack, pers. comm.). These results demonstrate that terminal differentiation in plants can proceed, at least to some degree, independently of cell division (Figure 2b). This conclusion is underscored by the observation that single-celled *Arabidopsis* trichomes can be forced to divide, giving rise to clustered and even multicellular leaf hairs that still display trichome characteristics [13–15].

Although terminally differentiated cells appear to tolerate cell divisions, strong induction of cell division during early leaf development by misexpressing *Arabidopsis CYCD3;1* or tobacco *CYCA3;2* under the *35S* promoter in *Arabidopsis* led to severe alterations of leaf morphology [16••,17••]. In both cases, the development of distinct spongy and palisade mesophyll layers was compromised and hyperproliferation occurred, especially in the epidermal layer. The epidermal cells of the *CYCD3;1*- or *CYCA3;2*-misexpression lines were small and polygonal, traits that are indicative of undifferentiated cells. Endoreplication, which is often associated with cell differentiation, was strongly inhibited in these lines. Two scenarios may account for these phenotypes. First, CYCA3;2 and CYCD3;1 might be special cyclins that are involved not only in controlling cell proliferation but also in repressing differentiation. Such an activity would have to be limited to only some cells because stomatal development was reported not to be strongly altered in the *CYCD3;1*-misexpression lines. Alternatively, rapid cell division during a

Figure 3



Many different cell-cycle modes are executed in plants. **(a)** The different cell-cycle modes can vary with respect to cell-cycle phase lengths, ranging from a rapid, proliferative mode to an exit from cell cycle in either G_1 or G_2. **(b)** The composition of different cell-cycle modes can also differ; for example, there is no mitosis in an endoreplicating mode.

critical point in development could interfere with the differentiation of some cells. The canonical pathway of D-type cyclin function results in the activation of a transcription factor, adenovirus E2 promoter binding factor (E2F), which in turn is involved in controlling entry into S-phase. Indeed, constitutive expression of *E2Fa* and its cofactor *DPa* (*DIMERIZATION PARTNERa*) from the *35S* promoter also led to a dramatic increase in cell number in both *Arabidopsis* and tobacco, suggesting that the primary differentiation defect caused by *35S:CYCD3;1* misexpression is due to strong promotion of cell proliferation [18•,19].

Taken together, this evidence indicates that proper cell divisions are crucial for differentiation programs (Figure 2a). However, there are different cell-cycle modes (based on different sets of regulators) that reflect developmental-time and cell-type-specific differences (Figure 3a). Developmental signals are required to install these different cell-cycle modes, underlining the importance of the developmental context (Figure 2b).

Cell cycle and cell morphogenesis

Specified cells often exit a mitotic cell-cycle mode and switch to an endoreplication (also called endoreduplication) program in which DNA replication is continued without a subsequent cell division (Figure 3b). In some cases, however, stimulation of cell divisions in already specified cells does not perturb cell morphogenesis (Figure 2c). For instance, the induction of cell divisions in endoreplicating trichomes, as found in the *siamese* mutant or in plants misexpressing B- or D-type cyclins, still allowed adoption of the general trichome morphology, including the initiation and expansion of branches (preferentially in the upper cells of a multicellular trichome) and the development of the characteristic cuticula with papillae [13–15]. Interestingly, several mutations that affect trichome branching appeared to have a function related to cell-cycle and/or cell-division control in other contexts [20]. This, together with the observation that trichomes are multicellular in many plant species, gave rise to speculation that *Arabidopsis* trichomes are derived from multicellular leaf hairs (presumably without endoreplication), and that the branching of *Arabidopsis* trichomes is a derivative of cell division (for further discussion see [20]). The origin of endoreplication from a mitotic mode could also explain why trichomes (and perhaps other endoreplicating cells) may tolerate ectopic cell divisions.

The potential advantages of a single large polyploid cell as opposed to many small cells might include facilitation of transport processes and the continuity of transcription as chromosomes do not condensate during endoreplication [21–23]. The recently described differences in the ranges of DNA movement in endoreplicated and diploid nuclei denote general changes in chromatin organization and structure that occur during endoreplication, and imply that vast changes in transcriptional control [24].

Furthermore, a correlation between nuclear size and cell size/cytoplasmic volume has often been observed [25]. This correlation is preserved in the aborted stomata found in CDKB1;1^{dn} plants, which displayed a 4C DNA content and were approximately the size of two guard cells each containing 2C [5••]. In addition, several *Arabidopsis* mutants that have altered trichomes support the correlation between cell size and nuclear size. Whereas wildtype trichomes have an DNA content of approximately 32C and develop mostly between three and four branches, mutants that have fewer endoreplication cycles have smaller trichomes with fewer branches [26]. Branch numbers and cell size are also increased in trichome mutants that have increased DNA contents [26]. Misexpression of a CDK inhibitor, and thus presumed direct interference with cell-cycle progression, concurrently reduced DNA levels and the size and branch number of trichomes. This represents another case, in which cell-cycle progression seems to direct the course of further differentiation

processes (Figure 2a; [27••]). Conversely, increased endoreplication in the trichomes of plants that misexpressed *CDC6* or *CDT1*, in which components of the replication machinery are again affected directly, was correlated with more branches and larger cells (Figure 2a; [4•]). The connection between cell size and DNA content seems to apply not only to terminally differentiated cells such as trichomes or guard cells. By applying inhibitors of DNA synthesis to *Arabidopsis* floral meristems and following their growth over time, a similar relationship between cell growth and DNA content could also be found in meristematic cells [28••].

Nevertheless, a few mutants and misexpression lines have been reported in which altered cell size and altered DNA content are not correlated. For instance, studies on a dominant-negative CDKA and on CDK inhibitors have shown that DNA content and cell size can be uncoupled, that is, that cells with less DNA than wildtype were larger than wildtype cells (Figure 2c; [11,29]). However, there are clearly limitations on the extent to which a cell can expand without an increase in DNA content, as seen in trichomes in which *INTERACTOR/INHIBITOR OF CDKs 1* (*ICK1*)/*KRP1* is misexpressed. Even though the ratio of cell size to DNA content to was much larger in these misexpression lines than in wildtype trichomes, the overall cell size of the transgenic trichomes was reduced in comparison to wildtype [27••].

Another example in which endoreplication appears to be required for proper cell differentiation is found in *Medicago* nodules, which are involved in nitrogen fixation. The anaphase-promoting complex/cyclosome (APC/C) activator protein CELL-CYCLE SWITCH 52 (CCS52; belonging to the CDH1, HCT1, FIZZY-RELATED [FZR] class) was previously shown to be required for endoreplication [30]. Downregulation of CCS52 resulted in smaller cells with reduced endoreplication levels, which failed to develop into N-fixing cells, and interestingly, died prematurely [31•]. As yet, it is not known how direct endoreplication is coupled to cell differentiation and cell survival, and/or whether other substrates of APC/C^{CCS52} are involved in nodule formation. However, *Arabidopsis* trichomes that were compromised in endoreplication because they misexpressed the CDK inhibitor *ICK1/KRP1* also underwent cell death, suggesting that endoreplication levels are directly involved in cell differentiation and cell survival (Figure 2; [27••]).

How endoreduplication, growth, and differentiation are mechanistically connected is not well understood. One possibility is that some regulators are involved in both processes at the same time (Figure 2d). For instance, the recent observation that CDKA;1 can be found *in vivo* in an complex with the translation initiation factor eIF4A offers such a link [32]. Another candidate for linking cell-cycle progression and differentiation is the group of E2F transcription factors. Misexpression of *E2Fa* together with *DPa* has been found to increase the level of endoreplicating cells [18•,19]. Interestingly, the transcription profiles of *E2F-DP* misexpressing plants and analysis of the E2F target genes revealed that E2F affects both genes that are involved in cell-cycle control and other target genes, including genes that have roles in nitrate assimilation and stress signalling [33•,34•]. Similarly, E2F appears to regulate many differentiation pathways in animals [35]. On a wider scale, recent expression profile studies in plants have shown that the expression of many genes that are involved in various cellular tasks, such as stress responses or metabolism, is specific to cell-cycle phase, indicating that many interrelationships between cell-cycle control and plant development are yet to be discovered [36•,37–39].

Cell cycle and tissue and organ growth

The most obvious link between cell cycle and differentiation at the organ level is found in meristems. Cell division and differentiation have to happen in a balanced manner for both the generation of new primordia and the maintenance of a stem-cell population (see recent reviews on meristem function [40–42]).

Furthermore, cell number and cell growth in the primordia and growing organs have to be regulated on a supercellular level to control organ and organism size. The regulation of organ growth is clearly comprised of two components: the cellular parameters, including cell size, cell shape and the division rate of individual cells; and non-cell-autonomous organ parameters, such as overall growth rate and growth direction. A long-range signal that functions in a non-cell-autonomous manner has recently been postulated subsequent to studies in which the growth dynamics of *Antirrhinum* petals were followed [43].

Compensation between cell size and cell number has been repeatedly observed as a part of a putative non-cell-autonomous surveillance function. In *Arabidopsis* plants that misexpresss *ICK2/KRP2* or $CDKB1;1^{dn}$, the leaves were comprised of fewer cells but these cells were much larger than those in wildtype plants. This finding was interpreted as an attempt to maintain overall organ size (Figure 2b; [5••,11]; for further review see Tsukaya [44]).

The ability to follow *in-vivo* cell division, cell enlargement, and differentiation is an important advance in the analysis of organ growth. Recently, Grandjean and colleagues [28••] adapted a special microscopical set-up to observe live *Arabidopsis* flower meristems, allowing the correlation of the expression of green fluorescent protein (GFP) reporter lines for primordia fate with cellular dynamics in these meristems. Two phases in primordia formation could be determined: first, a relatively rapid recruitment phase in which cells started to express the

GFP markers, followed by the onset of proliferation. Interestingly, interfering with cell division by applying the microtubule-depolymerising drug oryzalin in the recruitment phase blocked further development of primordia, whereas a later block did not prevent the outgrowth of primordia (by cell enlargement and endoreplication). Thus, a minimal number of cells is necessary to create an environment in which developmental cues are able to function (Figure 2a).

The recruitment of cells into primordia seems to be a non-cell-autonomous process and needs to be restricted by boundary regions in which cell proliferation is prevented. Breuil-Broyer and colleagues [45•] followed cell division in a semi-*in-vivo* culture system of cut flowers by labelling S-phase cells with 5-bromo-2-deoxyuridine (BrdU) and then determined the boundaries of non-dividing cells between growing primordia at cellular resolution. Remarkably, in the boundary region, no mRNAs could be found for either positive regulators of cell proliferation, such as *CYCD3;1*, or negative regulators, such as *ICK2/KRP2*, whereas both classes of genes were expressed in the surrounding primordia. In *Arabidopsis*, the *CUP-SHAPED COTELYDON* (*CUC*) genes, comprising a group of three highly homologous genes that encode putative transcription factors, appear to be involved in setting up boundaries in the meristems [46–48]. Mutations in *CUC* genes lead to partially fused organs and, in double or triple mutant combinations, to a loss of the shoot apical meristems. Whether and how directly *CUC* genes control cell-cycle regulators remains to be determined.

Even though Grandjean and colleagues [28••] observed that the individual cell-division rate can vary greatly in meristems, the overall pace of cell division appears to be highly regulated and to be important for development. In all attempts to increase plant growth reported to date, for instance in plants that misexpressed the recently identified *ARGOS* gene [49], the duration of cell division was temporally and spatially increased. By contrast, a great increase in the pace of cell division resulted in delayed differentiation, as seen in plants that misexpress *CYCA3;2*, *CYCD3;1*, or *E2Fa-DPa* [16••,17••,19]. In extreme cases, cell division activity came to halt and plant growth ceased. One interesting hypothesis to be tested in future is that a certain differentiation level is required to set the stage for cell proliferation.

Conclusions

Various relationships between cell-cycle control and differentiation occur; in addition, the succession of different relationships leads to complex developmental programs. In the generation of stomata, initial developmental signals are required for the formation of a meristemoid mother cell, which have to be coordinated with the general cell-division pattern in the leaf epidermis. Next, cell divisions appear to be required for further differentiation into a meristemoid cell, eventually leading to the formation of a guard mother cell. After this point, differentiation can proceed somewhat independently of cell-cycle progression. From an organ-level perspective, the number of meristemoid mother cells and satellite meristemoids is of primary importance as they will ultimately determine the number of guard cells, which will in turn control the balance between water loss and CO_2 uptake. The number of unequal cell divisions of the meristemoid also has to be neatly controlled on a supercellular level, because these divisions produce more than 70% of all epidermal cells, and thus are a major component of organ-size control [50].

Many of the observed relationships between cell cycle and plant development underline that plant cell-cycle control has a high degree of plasticity (e.g. already differentiated cells can re-enter a cell division cycle), and also possesses a high degree of robustness (e.g. terminal differentiation can proceed even though cell divisions continue). However, throughout development cell–cell communication seems to be required to set the stage for further cell-cycle progressions and differentiation.

An emerging theme is that cell-cycle regulators are cell-type specific or dependent on the developmental state. Thus, there is no definitive cell cycle but many different cell-cycle modes that are adapted for various settings. The organism might make use of these different cell-cycle modes by linking the expression and activity of other proteins to it. For instance, it has been speculated that HOBBIT, a subunit of the APC/C, is involved in mediating auxin responses in young and dividing cells, which of course have the greatest potential to influence the direction of plant growth and development [51]. Thus, we are just beginning to understand these different programs and how the plant uses them to regulate its development.

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- • of special interest
- •• of outstanding interest

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16. •• Dewitte W, Riou-Khamlichi C, Scofield S, Healy JM, Jacqmard A, Kilby NJ, Murray JA: **Altered cell cycle distribution, hyperplasia, and inhibited differentiation in *Arabidopsis* caused by the D-type cyclin CYCD3**. *Plant Cell* 2003, **15**:79-92.
Misexpression of *CYCD3;1* was found to strongly interfere with plant development, in a manner similar to that caused by the misexpression of *CYCA3;2* [17••]. In leaves, the development of distinct mesophyll layers was compromised and hyperproliferation, especially in the epidermal layer, was noted. In addition, epidermal cells displayed the characteristics of young and undifferentiated cells. This observation suggests that a great increase in cell proliferation inhibits differentiation. This is supported by the finding that plants that misexpress E2Fa-DPa, the target of the D-type cyclin pathway, displayed a similar phenotype [18•,19]. However, the *E2Fa-DPa* and *CYCD3;1* misexpression lines were not completely congruent and differed, especially with respect to endoreplication. In the *CYCD3;1* misexpression plants, the level of endoreplication was strongly reduced, whereas the *35S:E2F1-DPa* plants displayed increased levels of endoreplication, suggesting that at least CYCD3;1 has additional functions beyond the regulation of E2Fa.

17. •• Yu Y, Steinmetz A, Meyer D, Brown S, Shen WH: **The tobacco A-type cyclin, Nicta;CYCA3;2, at the nexus of cell division and differentiation**. *Plant Cell* 2003, **15**:2763-2777.
The authors describe the function of tobacco CYCA3;2. *CYCA3;2* transcripts were detected in organs with high proliferation activity, especially in flower buds. In contrast to many other cell-cycle genes, whose action was found to be buffered by redundantly working genes, an RNA interference (RNAi) construct against *CYCA3;2* caused defects in embryogenesis and inhibited callus regeneration. As no tobacco misexpression constructs of *CYCA3;2* could be recovered, a *35S:CYCA3;2* construct was transformed into *Arabidopsis* plants. These plants displayed similarities to *CYCD3;1* or *E2F-DP* misexpression plants, showing hyperproliferation and inhibition of cell differentiation [16••,18•,19]. Fluorescence-activated cell sorting (FACS) analysis showed that endoreplication was strongly reduced and most of the cells were found to be in a G_1 phase in these plants, indicating that *CYCA3;2* misexpression might guide cells through a complete cell-division cycle.

18. • Kosugi S, Ohashi Y: **Constitutive E2F expression in tobacco plants exhibits altered cell cycle control and morphological change in a cell type-specific manner**. *Plant Physiol* 2003, **132**:2012-2022.
The authors analyzed tobacco plants that misexpressed *Arabidopsis* E2Fa and DPa. Depending on the cell type, a strong increase in proliferation or an enhancement of endoreduplication levels was observed. Consistent with this, an induction of S-phase genes was also found. These phenotypes are similar to those seen in previous studies on E2Fa and DPa misexpression in *Arabidopsis* [19]. The effects in tobacco, however, were less dramatic and provided the opportunity to study the effect of E2Fa and DPa misexpression later in development; for instance, an altered flower morphology was found.

19. De Veylder L, Beeckman T, Beemster GT, de Almeida Engler J, Ormenese S, Maes S, Naudts M, Van Der Schueren E, Jacqmard A, Engler G *et al.*: **Control of proliferation, endoreduplication and differentiation by the *Arabidopsis* E2Fa-DPa transcription factor**. *EMBO J* 2002, **21**:1360-1368.

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21. Sugimoto-Shirasu K, Roberts K: **"Big it up": endoreduplication and cell-size control in plants**. *Curr Opin Plant Biol* 2003, **6**:544-553.

22. Kondorosi E, Roudier F, Gendreau E: **Plant cell-size control: growing by ploidy?** *Curr Opin Plant Biol* 2000, **3**:488-492.

23. Larkins BA, Dilkes BP, Dante RA, Coelho CM, Woo Ym Y, Liu Y: **Investigating the hows and whys of DNA endoreduplication**. *J Exp Bot* 2001, **52**:183-192.

24. Kato N, Lam E: **Chromatin of endoreduplicated pavement cells has greater range of movement than that of diploid**

guard cells in *Arabidopsis thaliana*. *J Cell Sci* 2003, **116**:2195-2201.

25. Nagl W: *Zellkern und Zellzyklen: Molekularbiologie, Organisation und Entwicklungsphysiologie der Desoxyribonucleinsaeure und des Chromatins*. Stuttgart: Ulmer; 1976. [Title translation: Cell nucleus and cell cycles: molecular biology, organisation and development of DNA and chromatin.]

26. Hülskamp M, Schnittger A, Folkers U: **Pattern formation and cell differentiation: trichomes in *Arabidopsis* as a genetic model system**. *Int Rev Cytol* 1999, **186**:147-178.

27. •• Schnittger A, Weinl C, Bouyer D, Schobinger U, Hülskamp M: **Misexpression of the cyclin-dependent kinase inhibitor ICK1/KRP1 in single-celled *Arabidopsis* trichomes reduces endoreduplication and cell size and induces cell death**. *Plant Cell* 2003, **15**:303-315.

Using *Arabidopsis* trichomes, the authors analyzed the relationship between cell growth and DNA content independently of the influence of organ-size control. The study revealed that trichome growth is regulated by DNA-dependent as well as DNA-independent growth mechanisms. Furthermore, it provided evidence that CDK inhibitors control cell survival. Yet, it is not known whether this function is a direct feature of CDK inhibitors or results from a compromised endoreplication cycle or a distorted relationship between cell size and DNA content in trichomes.

28. •• Grandjean O, Vernoux T, Laufs P, Belcram K, Mizukami Y, Traas J: ***In vivo* analysis of cell division, cell growth, and differentiation at the shoot apical meristem in *Arabidopsis***. *Plant Cell* 2004, **16**:74-87.

The authors were able to follow *in vivo* cell division and differentiation in *Arabidopsis* shoot meristems. Pin-formed meristems were created by applying the auxin transport inhibitor nitropropionic acid (NPA), which blocks the generation of primordia so that no old primordia would shield the development of new primordia. Upon release from the NPA regime, cell division and the expression of GFP–promoter reporter lines were monitored. In addition, the effects of inhibitors of cell division (i.e. oryzalin) and DNA synthesis (i.e. aphidicolin and hydroxy urea) on meristems were observed.

29. Hemerly A, Engler J, Bergounioux C, Van Montagu M, Engler G, Inzé D, Ferreira P: **Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development**. *EMBO J* 1995, **14**:3925-3936.

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31. • Vinardell JM, Fedorova E, Cebolla A, Kevei Z, Horvath G, Kelemen Z, Tarayre S, Roudier F, Mergaert P, Kondorosi A *et al.*: **Endoreduplication mediated by the anaphase-promoting complex activator CCS52A is required for symbiotic cell differentiation in *Medicago truncatula* nodules**. *Plant Cell* 2003, **15**:2093-2105.

The authors analyzed the function of the APC/C activator protein CCS52A in regulating endoreplication cycles in *Medicago* nodule cells and the implications of this role for nodule formation. Expression of an antisense construct against CCS52A in nodule primordia decreased cell size and reduced the number of endoreplication cycles, underlining the correlation between DNA content and cell size. Interestingly, cells that had compromised endocycles were also blocked in further differentiation into nitrogen-fixing cells and died prematurely.

32. Hutchins AP, Roberts GR, Lloyd CW, Doonan JH: ***In vivo* interaction between CDKA and eIF4A: a possible mechanism linking translation and cell proliferation**. *FEBS Lett* 2003, **556**:91-94.

33. • Vlieghe K, Vuylsteke M, Florquin K, Rombauts S, Maes S, Ormenese S, Van Hummelen P, Van de Peer Y, Inzé D, De Veylder L: **Microarray analysis of E2Fa-DPa-overexpressing plants uncovers a cross-talking genetic network between DNA replication and nitrogen assimilation**. *J Cell Sci* 2003, **116**:4249-4259.

Transcript profiling was carried out on plants that concurrently overexpressed E2Fa and DPa. The profiles showed the upregulation of genes that are involved in DNA replication and modification and, unexpectedly, of genes that are required for nitrogen assimilation and photosynthesis. These profiles suggest that cell proliferation has high nitrogen and energy demands. Analysis of the promoters of identified target genes, however, showed no clear correlation between the occurrence of a canonical E2F-consensus binding site and the induction of gene expression by E2Fa-DPa.

34. • Ramirez-Parra E, Frundt C, Gutierrez C: **A genome-wide identification of E2F-regulated genes in *Arabidopsis***. *Plant J* 2003, **33**:801-811.

The authors of this paper describe the *in-silico* identification of putative E2F-binding sites in *Arabidopsis* promoters, and an expression analysis of a selected set of putative E2F target genes in a semi-synchronized cell culture. Putative E2F target genes were further studied in plants that misexpressed a dominant-negative *DP* gene. This work highlights the importance of E2F factors for the expression of cell-cycle and transcription-factor genes.

35. Dimova DK, Stevaux O, Frolov MV, Dyson NJ: **Cell cycle-dependent and cell cycle-independent control of transcription by the *Drosophila* E2F/RB pathway**. *Genes Dev* 2003, **17**:2308-2320.

36. • Menges M, Hennig L, Gruissem W, Murray JA: **Genome-wide gene expression in an *Arabidopsis* cell suspension**. *Plant Mol Biol* 2003, **53**:423-442.

Arabidopsis cell-suspension cultures were synchronized and expression profiles were analyzed at different phases of a successive cell cycle. After careful reassessment, more than 1000 genes were identified as being cell-cycle regulated, including genes encoding NAC and MADS-box transcription factors. These genes represent a plethora of yet-to-be-analyzed connections between cell-cycle regulation and plant development.

37. Hennig L, Menges M, Murray JA, Gruissem W: ***Arabidopsis* transcript profiling on Affymetrix GeneChip arrays**. *Plant Mol Biol* 2003, **53**:457-465.

38. Breyne P, Dreesen R, Cannoot B, Rombaut D, Vandepoele K, Rombauts S, Vanderhaeghen R, Inzé D, Zabeau M: **Quantitative cDNA-AFLP analysis for genome-wide expression studies**. *Mol Genet Genomics* 2003, **269**:173-179.

39. Breyne P, Dreesen R, Vandepoele K, De Veylder L, Van Breusegem F, Callewaert L, Rombauts S, Raes J, Cannoot B, Engler G *et al.*: **Transcriptome analysis during cell division in plants**. *Proc Natl Acad Sci USA* 2002, **99**:14825-14830.

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41. Doerner P: **Plant meristems: a merry-go-round of signals**. *Curr Biol* 2003, **13**:R368-R374.

42. Carles CC, Fletcher JC: **Shoot apical meristem maintenance: the art of a dynamic balance**. *Trends Plant Sci* 2003, **8**:394-401.

43. Rolland-Lagan A, Bangham J, Coen E: **Growth dynamics underlying petal shape and asymmetry**. *Nature* 2003, **422**:161-163.

44. Tsukaya H: **Organ shape and size: a lesson from studies of leaf morphogenesis**. *Curr Opin Plant Biol* 2003, **6**:57-62.

45. • Breuil-Broyer S, Morel P, de Almeida-Engler J, Coustham V, Negrutiu I, Trehin C: **High-resolution boundary analysis during *Arabidopsis thaliana* flower development**. *Plant J* 2004, **38**:182-192.

The authors describe the application of 5-bromo-2-deoxyuridine (BrdU) labelling for the determination of S-phase cells in the *Arabidopsis* inflorescences. They correlate the resulting pattern of S-phase cells with the expression of several cell-cycle genes. The expression of both positive and negative regulators of cell-cycle progression was found in regions of rapid cell proliferation, which were identified by BrdU incorporation. These regions were clearly separated by BrdU negative cells, in which transcripts of neither positive nor negative cell-cycle regulators were detected. The expression of the *CUP-SHAPED COTELYDON2* gene in these non-cycling cells highlights the importance of the boundary genes for proper meristem function and organ development.

46. Vroemen CW, Mordhorst AP, Albrecht C, Kwaaitaal MA, de Vries SC: **The *CUP-SHAPED COTYLEDON3* gene is required for boundary and shoot meristem formation in *Arabidopsis***. *Plant Cell* 2003, **15**:1563-1577.

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