Cell cycle controls and the development of plant form

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The relationship between cell division and plant form has long been a battleground for the debate between those proclaiming and disclaiming an important role for cell division in morphogenetic and developmental processes. Recent evidence suggests that cell division and morphogenesis are intimately interconnected, and whereas overall architecture is determined by patterning genes, the elaboration and execution of developmental programmes require proper control of the cell-division cycle.

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Abbreviations

ANT	*AINTEGUMENTA*
APC	anaphase-promoting complex
ccs52	*cell cycle switch 52*
cdc25	cell-division cycle protein 25
CDK	cyclin-dependent kinase
CycD	D-type cyclin
ICK1	interactor of cdc2 kinase 1
pRb	retinoblastoma protein
rml1	*rootmeristemless1*
STM	*SHOOT MERISTEMLESS*

Introduction

Cell division without patterning produces disorganised callus tissue, whereas higher plants develop from a single-celled zygote into a multicellular organism through co-ordinated cell divisions [1,2]. Polarised forces establish the root and shoot meristems (i.e. the growth points in plants) early in the development of the embryo. These meristem cells and their descendants give rise to the various tissues and organs of a mature plant through the combined processes of cell division, cell expansion and cell differentiation.

The immobility of both plants and their constituent cells suggests that co-ordinated control of cell division is likely to be important in both environmental responses and developmental processes. To what extent is this true? The argument has tended to be clouded by polarised views based on cell and organism perspectives, encapsulated in the aphorism attributed to de Bary [3]: "Die Pflanze bildet Zellen, nicht die Zelle bildet Pflanzen" (The plant forms cells, not cells the plant).

Thus, in the one extreme of the organismal theory of development, cell division is seen as a mechanism for sub-dividing the volume of the organism into conveniently managed units. The processes of enlargement, differentiation and formation of tissues are regarded as essentially independent of cell division, which is simply a necessary consequence of their execution [4]. This is in contrast to the cell theory of development, which states that cellular behaviour is the major factor in determining developmental processes [5].

Here, we briefly review the plant cell cycle to provide the background for considering the different perspectives on its role, and argue for an integrated view of cell division in development. We consider data that suggest developmental roles for cell cycle genes and discuss a number of developmental changes that demonstrate specific roles for the cell-division cycle in controlling the development of plant form.

Cell-division cycle

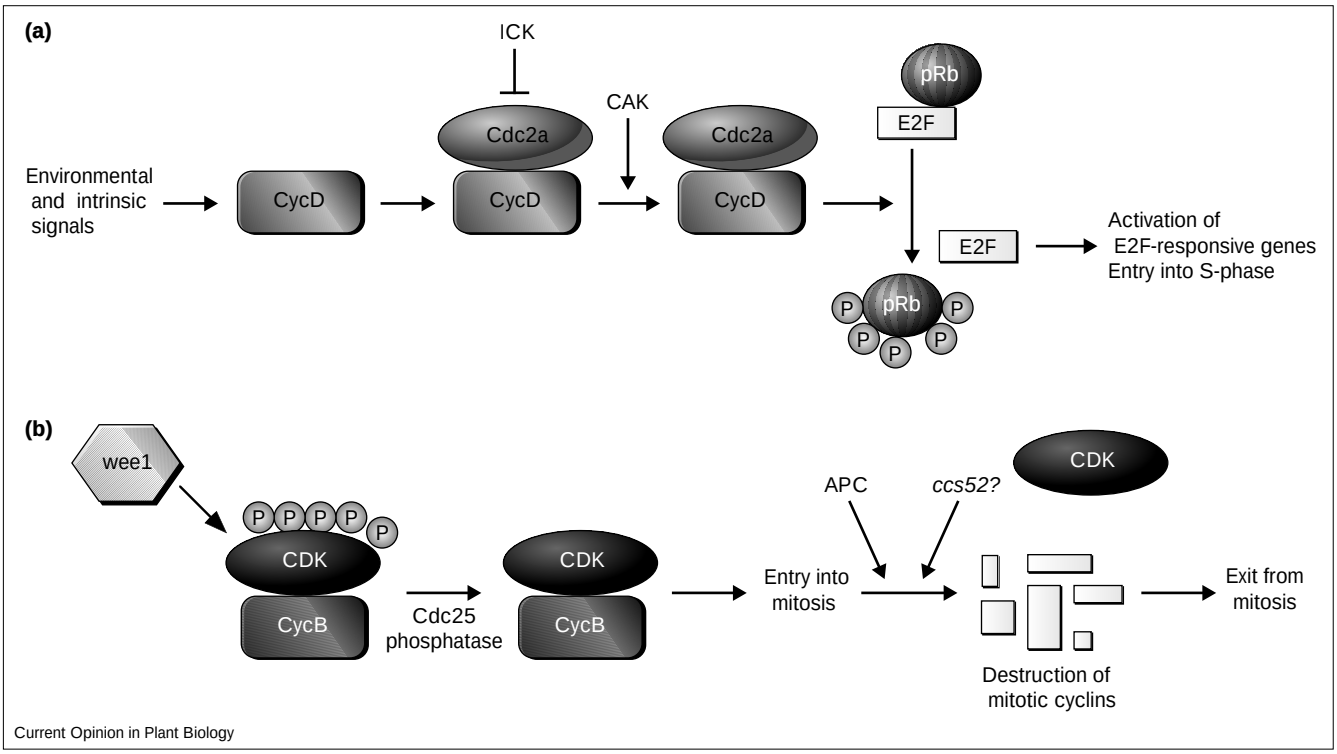
Cell division is a basic characteristic of all living organisms. In eukaryotes, the DNA replication phase (i.e. the S phase) is separated from the cell-division phase (i.e. the M phase) by two gap phases (G_1 and G_2) in the sequence G_1–S–G_2–M [6].

In all eukaryotes, cell cycle regulation depends on cyclin-dependent kinases (CDKs) complexed with their associated cyclins at both the $G_1 \rightarrow S$ phase and the $G_2 \rightarrow M$ phase transitions. These are probably the major control points in the cell cycle at which decisions are taken with respect to division, differentiation, programmed cell death, or adoption of a quiescent state (G_0) [7]. Excellent reviews of the plant cell cycle have appeared recently [8,9,10••], so here, we will only briefly outline the likely molecular mechanisms of plant cell cycle control.

As mentioned above, key checkpoints in cell cycle control operate at the $G_1 \rightarrow S$ and $G_2 \rightarrow M$ transitions, and involve two major classes of CDK (CDK-a and CDK-b; [9,10••,11]), which differ in their cyclin-binding motifs. During G_1, environmental and intrinsic signals result in an increase in D-type cyclin (CycD) levels, these cyclins associate with CDK-a (Figure 1; [12•,13••,14]). The activity of these CDK–CycD complexes may be regulated by CDK inhibitor proteins, such as ICK1 (interactor of cdc2 kinase 1) [15] and ICK2 [16]. The current model proposes that the increased activity of CycD–CDK in late G_1 phosphorylates the retinoblastoma protein (pRb), thereby inactivating it and releasing E2F transcription factors from pRb repression [17]. E2F-responsive genes are then transcribed and the cells enter the S phase. Progression through the S phase is probably controlled by cyclinA (CycA) kinases [18].

At the $G_2 \rightarrow M$ transition, cyclinB–CDK complexes are initially inactive because of inhibitory phosphorylation carried out by the wee1 kinase [19], but are activated, presumably by homologues of the cdc25 (cell-division cycle protein 25) phosphatase [20], after which the cells enter mitosis. The

Figure 1



Model of key checkpoints during cell cycle progression in plants. (a) Activation of G_1 progression and $G_1 \rightarrow S$ control. Environmental and intrinsic signals result in an increase in CycD, which associate with Cdc2a. The activity of the complex is potentially modulated by ICKs and CDK activating kinases (CAKs) [53]. Late in G_1, the increased levels of CycD–CDK phosphorylate (represented by P) the pRb, releasing E2F transcription factors from repression by pRb [54]. The activation of the E2F-responsive genes results in progression into S-phase. (b) $G_2 \rightarrow M$ transition. CycB–CDK complexes, which are initially inactive because of their phosphorylation by the wee1 kinase, are activated by the Cdc25 phosphatase, causing the cells to enter mitosis. Exit from mitosis requires the destruction of mitotic cyclins by the APC and possibly *ccs52* [22••].

involvement of other protein phosphatases in co-ordinating chromosomal and microtubule events has also been proposed [21]. Exit from mitosis requires the destruction of mitotic cyclins by the anaphase-promoting complex (APC) and possibly a role for *ccs52* (*cell cycle switch 52*), a gene related to *Fizzy-related* of *Drosophila*, in the switch from cell-division cycle to endoreduplication cycle [22••].

Meristems, organogenesis and cell division

Apparent support for the organismal theory of development in plants comes from early research with γ-irradiated wheat seedlings, which show the outgrowth of a fourth leaf primordium when cell division is arrested [23–25]. This protrusion is, however, limited in size and appears abnormal. No stomata or trichomes develop on this 'leaf' and further development is arrested. Although often cited as providing support for the view that shape and form are acquired independently of cell division, we would rather argue that these results show that cell division is essential for development in any meaningful sense. These data are easily rationalised as a consequence of the uncoupling of growth from division, with the continuation of polarised growth leading to the protrusion. Alternatively, if we accept Green's position [26] that cell division and cell expansion are different phases of a continuous process, then we see the continued 'growth' of such primordia as the completion of the expansion phase of cells already formed at the time of irradiation. Cell division should not, therefore, be disregarded simply as a secondary consequence of growth, as both cell division and cell expansion contribute to growth and are needed for normal growth and elaboration of structure.

The principal sites of cell division in plants are meristems [27]. The *rootmeristemless1* (*rml1*) mutant has recently provided evidence of the essential requirement for cell division in development. In the postembryonic root meristem of this mutant, a specific cell cycle arrest in G_1 is caused by the loss of γ-glutamylcysteine synthase, the first enzyme of glutathione biosynthesis [28•]. *rml1* mutants have normal axial and radial patterning, but cannot maintain an undifferentiated meristematic zone. Although unrelated to *RML1*, the *SHOOT MERISTEMLESS* (*STM*) gene is similarly required for the establishment of the postembryonic shoot meristem and for the maintenance of the undifferentiated state of its cells [27,29•]. *STM* may prevent the differentiation of meristem cells by promoting their proliferation. Thus, not only may meristematic function be intimately linked with cell division, but it is also

quite possible that the maintenance of meristem cells in an undifferentiated state is, itself, dependent on their continued proliferation. If this is true, cell division is not simply a function of meristems, but may control their identity in a profound sense, as it determines their continued existence.

Cell division and embryogenesis

Laser-ablation experiments have shown that position, rather than lineage, determines patterning and cell fate in roots (see below). This evidence suggests that patterns formed early in embryogenesis are likely to be important in determining later developmental form. The higher-plant zygote undergoes an initial asymmetric cell division (reviewed in [30]), which establishes the polarity that probably determines subsequent pattern formation [31]. *Arabidopsis* mutants such as *monopteros* and *gnom* show abnormal patterns of cell division in the early embryo, suggesting that pattern formation may be dependent on division planes. Are subsequent divisions important for the form of the embryo? Although, in *Arabidopsis*, the pattern of cell division in the embryo is indeed very regular and seedling structures can be traced back to groups of cells in the early embryo [32], this is not common to all plants [33]. It has been argued, therefore, that the embryonic pattern mutants of *Arabidopsis* are related to the size of the *Arabidopsis* plant and its uniform growth. The phenotype of the *fass* mutant supports this conclusion, as *fass* mutations result in disorganised cell divisions giving rise to an embryo that is normally patterned, in the sense that all elements of the body pattern are formed [32]. Despite the successful establishment of cell types in *fass* mutants, however, the plants are severely dwarfed, have organs that do not elongate correctly and lack many histological features, such as trichomes. Thus, although embryonic patterning may not involve specific cell divisions, final form does.

The problem with interpreting the role of cell division in mutant phenotypes such as that of *fass* is that, depending on the genetic lesion, cell division may be affected only indirectly. Using a transgenic approach involving the expression of a dominant negative version of CDK-a *cdc2aDN*, which inhibits cell division, a variety of phenotypes were observed in embryos [34••]. In the most severe cases, basic tissue organisation could not be recognised, whereas in others, certain tissues were missing and distortions of apical–basal pattern were seen, although radial patterning was normal. In embryos in which tissues were present, their cells adopted correct characteristics, again showing that position determines cell fate. These results therefore show that perturbation of normal cell-division rates during embryogenesis disturbs apical–basal morphogenesis.

Leaf development

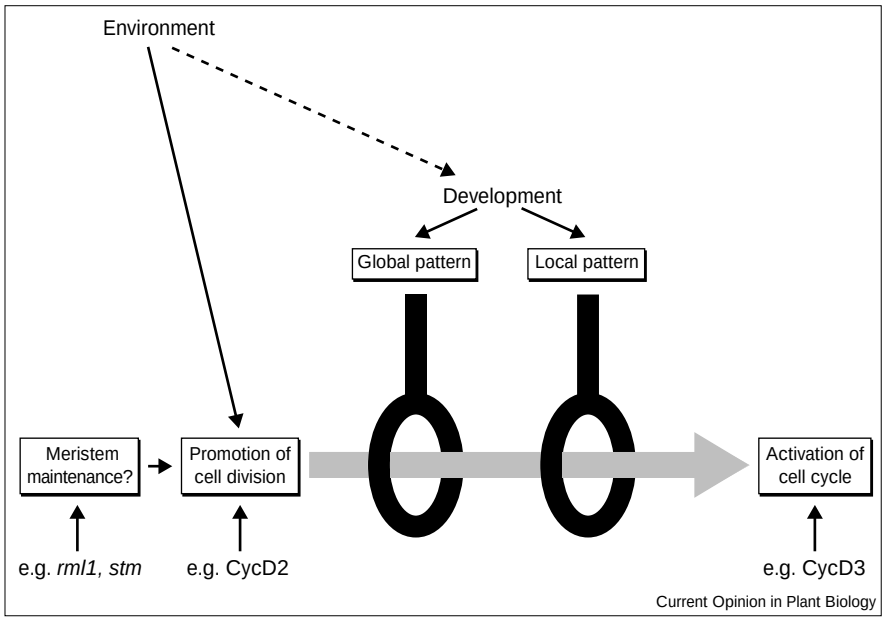
In contrast to apical meristems, which have an indeterminate growth plan, the leaves of higher plants have determinate growth with a fixed period of development [1]. Leaf-blade inception is associated with cycling activity in the 'axillary meristem', a group of small cells with densely packed cytoplasm and small vacuoles, which is located between the adaxial and abaxial sides of the leaf [35•]. The use of a β-glucuronidase (GUS)-reporter gene fused to a mitotic cyclin revealed that blade formation is highly correlated with localised cell cycling, and that different tissue layers follow complex patterns of division [36••]. Developing trichome cells undergo endoreduplication while the surrounding trichome support cells continue to divide ([36••], reviewed in [37]). Precise control of cell division is also found during the formation of stomatal guard cells, and separation of adjacent guard-cell pairs is achieved through a series of regulated asymmetric cell divisions. Despite this correlative evidence for the necessity for cell division in some aspects of leaf development, a substantial body of evidence from chimera analysis in leaves shows that the contribution of specific cells and layers within the meristem to particular regions of the leaf is variable [38]. The maize *tangled-1* mutant has abnormally oriented cell divisions, yet develops a normal leaf with normal cell layers, albeit with roughened texture and smaller size than a wild-type leaf [39]. Leaf development in this mutant is, therefore, consistent with an overall pattern that is largely independent of particular cell divisions. As in *fass* mutant leaves, however, *tangled-1* leaves have histological abnormalities in their stomata and hair distribution and in their venation patterns.

Does disruption of normal cell division affect form? Inhibition of cell division in tobacco using the *cdc2aDN* allele discussed above, resulted in leaves with normal shape but smaller overall size, which contained fewer but larger cells [40]. As we might have predicted, altering the rate of cell division in these leaves does not affect the execution of patterning genes. Increased cell size may be viewed either as resulting from the uncoupling of cell growth and cell division or as a direct correction by the morphogenetic programme to compensate for the lack of cells. As most yeast cell cycle mutants have large cells, resulting from the uncoupling of cell growth and cell division, we favour the former interpretation.

Transgenic plants with increases in the levels of two different D-type cyclins, which promote cell division during the G_1 phase of the cell cycle, have recently been reported to have strikingly different consequences [13••,41••]. CycD3 responds to phytohormones, particularly cytokinins, and its overexpression results in the formation of abnormal *Arabidopsis* plants that have leaves curled along their proximal–distal axis and that contain numerous small, incompletely differentiated cells ([13••]; W Dewitte, C Riou-Khamlichi, JAH Murray, unpublished data). In contrast, CycD2 overexpression in tobacco increases the rate of cell division and overall plant growth without affecting morphology [41••]. Thus, CycD2 appears to promote cell division in such a way that it is still subject to pattern controls, whereas CycD3 is able to act like an oncogene and directly drive cell division downstream of normal developmental and patterning controls (Figure 2).

Figure 2

The hierarchy of controls operating on cell division provides a framework for understanding the phenotypes observed from the disruption of cell-division controls. The rate of cell division may be primarily under environmental control, and is interpreted through the filters of global- and local-patterning controls. Thus, increasing cell division rate by overexpression of CycD2 does not alter normal patterning. Directly triggering inappropriate cell divisions 'downstream' of patterning controls may, however, disrupt either global or local morphogenesis (e.g. in CycD3 overexpressing plants). Formation and maintenance of meristems may also be dependent on the rate of cell division, as there is a correlation between continued cell division and continued meristem function in mutants such as *rml1* and *stm*.



AINTEGUMENTA (*ANT*) regulates cell numbers during organogenesis [42••]. Plants with reduced *ANT* levels, like *cdc2aDN* plants, have smaller than normal organs with fewer- but larger-than-normal cells. Overexpression of *ANT* produces enlarged organs with superficially normal morphology and normal-sized cells. *ANT* appears, therefore, to prolong the period in which cells maintain division competence, but without disrupting pattern controls.

Root development

In *Arabidopsis* roots, each initial cell from the root apical meristem has a stereotypical pattern of cell division that leads to each column in the root [43]. Laser-ablation studies of initial cells have shown that pattern formation in the root is controlled by short-range signals that inhibit differentiation and by signals that reinforce cell fate [44]. Position, not lineage, determines cell behaviour.

Transgenic tobacco plants expressing the fission yeast gene *cdc25*, which would be expected to accelerate the entry of cells into mitosis by activating CDKs, showed the initiation of abnormally high numbers of lateral roots, which had significantly smaller-than-normal cells [45]. These findings contrast with those of Doerner *et al.* [46] who reported that modest overexpression of the mitotic B-type cyclin *CycB1;1*, under the control of a *cdc2aAt* promoter, increased the rate of root growth without changing cell size or the number of lateral root primordia. The induction of CycD4;1 expression by mitogenic signals, such as sucrose, in *Arabidopsis* lateral root primordia might be one of the rate-limiting steps in the initiation of lateral roots [47]. The patterns of accumulation of different mitotic cyclins in maize roots during cell differentiation and lateral root induction suggest that different cyclins have specific roles in controlling these processes [48•].

Floral development

Flowering plant morphogenesis depends almost entirely on the control of the pattern and rate of cell division. Many morphological mutants in which cell division is, most often indirectly, affected have been identified [49]. One interesting example is *tousled*, a mutant affected in a gene that encodes a novel type of serine/threonine kinase whose human homologue was subsequently found to be cell cycle regulated during DNA replication [50].

In situ hybridisation studies on *Antirrhinum majus* inflorescences showed that different genes of the *CycD3* group show differential expression [12•]. CycD cyclins are modulated by plant growth substances [12•,13••,14] and are thought to be important for signal transduction between the environment and the cell cycle machinery [51]. During vegetative and floral development, *CycD3a* expression is restricted to the organ primordia, whereas *CycD3b* expression occurs in all dividing cells. Moreover, repression of *CycD3b* expression by the *Cycloidea* gene is essential for normal floral development [12•].

Conclusions: the plant makes cells and the cells make the plant

Cell cycle controls are subject to both rate and patterning regulation. Patterning genes define the desired final form of plants and their constituent organs, but properly controlled cell division is essential for executing the blueprint. Thus, overall organ development and form is dependent neither on cell lineage nor on particular patterns or rates of cell division but, nevertheless, requires spatial and developmental control of cell division and its integration with cell differentiation.

Cell cycle control of particular divisions in local domains appear to be essential for (and perhaps a driving force for)

the establishment of specific histological structures, such as stomata, leaf veins, root galls and trichomes. Additionally, overall rates of cell division may determine quantitative responses to the environment. Although final plant shape may be fixed, plants often respond to fluctuations in their environment by changes in cell-division rates in meristems [52].

Alteration of normal cell division can therefore variously affect either local and global morphogenesis or rates of cell division. The phenotypic effects of mutations that disrupt cell division will depend on the nature of the lesion, particularly on whether it acts downstream of patterning regulation or prevents its implementation.

We speculate that continued cell division may be essential for maintaining the undifferentiated state of meristem cells. Relative rates of division in different regions of the shoot meristem may also be important in maintaining its normal structure and function.

We conclude that there are complex interactions between morphogenetic and cell-division pathways. The cell cycle is neither slave nor master of development, but is integrated into complex pathways of morphogenesis and histogenesis. The cell cycle actively responds to environmental stimuli and adapts the rate and orientation of cell divisions accordingly. The major patterning genes responsible for plant form, as well as the main players in cell cycle control, have now been identified. In the future, exciting new insights into how cell division is integrated within the unique developmental contexts of plants will be revealed.

Acknowledgements

We apologise to authors whose work is not discussed or explored in more detail because of lack of space.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lyndon RF: *The Shoot Apical Meristem – Its Growth and Development*. Cambridge University Press; 1998.

2. Steeves TA, Sussex IM: *Patterns in Plant Development*, edn 2. Cambridge University Press; 1989.

3. Barlow PW: 'The plant forms cells, not cells the plant': the origin of de Bary's aphorism. *Ann Botany* 1982, 49:269-271.

4. Kaplan DR, Hagemann W: The relationship of cell and organism in vascular plants – are cells the building blocks of plant form? *BioScience* 1991, 41:693-703.

5. Evered D, Marsh J: *The Cellular Basis of Morphogenesis.* Chichester, UK: John Wiley; 1989.

6. Howard A, Pelc SR: Synthesis of deoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. *Heredity* 1953, 6(suppl):216-273.

7. Reichheld JP, Chaubet N, Shen WH, Renaudin JP, Gigot C: Multiple A-type cyclins express sequentially during the cell cycle in ***Nicotiana tabacum*** BY2 cells. *Proc Natl Acad Sci USA* 1996, 93:13819-13824.

8. Den Boer BG, Murray JAH: Triggering the cell cycle in plants. *Trends Cell Biol* 2000, 10:245-250.

9. Huntley RP, Murray JAH: The plant cell cycle. *Curr Opin Plant Biol* 1999, 2:440-446.

10. •• Mironov V, De Veylder L, Van Montagu M, Inzé D: Cyclin-dependent kinases and cell division in plants – the nexus. *Plant Cell* 1999, 11:509-521.
An excellent and comprehensive review of the control of the plant cell cycle.

11. Umeda M, Umeda-Hara C, Yamaguchi M, Hashimoto J, Uchimiya H: Differential expression of genes for cyclin-dependent protein kinases in rice plants. *Plant Physiol* 1999, 119:31-40.

12. • Gaudin V, Lunness PA, Fobert PR, Towers M, Riou-Khamlichi C, Murray JA, Coen E, Doonan JH: The expression of D-cyclin genes defines distinct developmental zones in snapdragon apical meristems and is locally regulated by the ***Cycloidea*** gene. *Plant Physiol* 2000, 122:1137-1148.
The authors demonstrate that *Antirrhinum* has two different genes encoding CycD3 cyclins. Interestingly, these two genes show striking tissue specificity in their expression. *CycD3b* transcripts are found in all dividing cells, whereas *CycD3a* transcripts are found only in organ primordia.

13. •• Riou-Khamlichi C, Huntley R, Jacqmard A, Murray JAH: Cytokinin activation of ***Arabidopsis*** cell division through a D-type cyclin. *Science* 1999, 283:1541-1544.
CycD3 of *Arabidopsis* is shown to be induced by the phytohormone cytokinin, and plants constitutively expressing CycD3 can produce callus in the absence of exogenous cytokinin. This finding suggests that CycD3 is important in mediating cytokinin effects in the control of the $G_1 \rightarrow S$ transition.

14. Riou-Khamlichi C, Menges M, Healy JM, Murray JA: Sugar control of the plant cell cycle: differential regulation of ***Arabidopsis*** D-type cyclin gene expression. *Mol Cell Biol* 2000, 20:4513-4521.

15. Wang H, Qi Q, Schorr P, Cutler AJ, Crosby WL, Fowke LC: ICK1, a cyclin-dependent protein kinase inhibitor from ***Arabidopsis thaliana*** interacts with both Cdc2a and CycD3, and its expression is induced by abscisic acid. *Plant J* 1998, 15:501-510.

16. Lui H, Wang H, Delong C, Fowke LC, Crosby WL, Fobert PR: The ***Arabidopsis*** Cdc2a-interacting protein ICK2 is structurally related to ICK1 and is a potent inhibitor of cyclin-dependent kinase activity ***in vitro***. *Plant J* 2000, 21:379-385.

17. De Jager SM, Murray JA: Retinoblastoma proteins in plants. *Plant Mol Biol* 1999, 41:295-299.

18. Roudier F, Fedorova E, Gyorgyey J, Feher A, Brown S, Kondorosi A, Kondorosi E: Cell cycle function of a ***Medicago sativa*** A2-type cyclin interacting with a PSTAIRE-type cyclin-dependent kinase and a retinoblastoma protein. *Plant J* 2000, 23:73-83.

19. Sun Y, Dilkes BP, Zhang C, Dante RA, Carneiro NP, Lowe KS, Jung R, Gordon-Kamm WJ, Larkins BA: Characterization of maize (***Zea mays*** L.) Wee1 and its activity in developing endosperm. *Proc Natl Acad Sci USA* 1999, 96:4180-4185.

20. Millar JBA, Russell P: The cdc25 M-phase inducer: an unconventional protein phosphatase. *Cell* 1992, 68:407-410.

21. Ayaydin F, Vissi E, Meszaros T, Miskolczi P, Kovacs I, Feher A, Dombradi V, Erdodi F, Gergely P, Dudits D: Inhibition of serine/threonine-specific protein phosphatases causes premature activation of cdc2MsF kinase at G_2/M transition and early mitotic microtubule organisation in alfalfa. *Plant J* 2000, 23:85-96.

22. •• Cebolla A, Vinardell JM, Kiss E, Olah B, Roudier F, Kondorosi A, Kondorosi E: The mitotic inhibitor ccs52 is required for endoreduplication and ploidy-dependent cell enlargement in plants. *EMBO J* 1999, 18:4476-4484.
ccs52 is a plant homologue of the APC activators that are required for mitotic cyclin destruction. In *Medicago*, however, increased levels of *ccs52* RNA were present in endoreduplicating cells, and antisense downregulation of *ccs52* decreased the number of rounds of endoreduplication and the size of the largest cells. The authors suggest a role for *ccs52* in switching proliferating cells to endocycling cells.

23. Foard DE: The initial protrusion of a leaf primordium can form without concurrent periclinal cell divisions. *Can J Botany* 1971, 49:1601-1603.

24. Haber AH: Non-essentiality of concurrent cell divisions for degree of polarisation of leaf growth. I. Studies with radiation-induced mitotic inhibition. *Amer J Botany* 1962, 49:583-589.

25. Haber AH, Carrier WL, Foard DE: Metabolic studies of gamma-irradiated wheat growing without cell division. *Am J Botany* 1961, 48:431-438.

26. Green PB: Growth and cell pattern formation on an axis: critique of concepts, terminology, and modes of study. *Bot Gaz* 1976, 137:187-202.

27. Laufs P, Grandjean O, Jonak C, Kieu K, Traas J: Cellular parameters of the shoot apical meristem in ***Arabidopsis***. *Plant Cell* 1998, 10:1375-1390.

28. • Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S, Brown S, Maughan SC, Cobbett CS, Van Montagu M, Inze D *et al.*: ***The ROOT MERISTEMLESS1/CADMIUM SENSITIVE2*** gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *Plant Cell* 2000, 12:97-110.
The *RML* gene is shown to encode the first enzyme of glutathione biosynthesis. Mutation in the *RML1* gene causes arrest of root meristem cells specifically in G_1, as do inhibitors of glutathione biosynthesis. The arrested root meristem cells differentiate; one of several possible explanations for this is that continued cell division is required to prevent the differentiation of meristem cells.

29. • Hemerly AS, Ferreira PCG, Van Monague M, Inze D: Cell cycle control and plant morphogenesis: is there an essential link? *BioEssays* 1999, 21:29-37.
A review of the relationships between cell cycle control and plant morphogenesis.

30. Scheres B, Benfry BN: Asymmetric cell division in plants. *Annu Rev Plant Phys Plant Mol Biol* 1999, 50:505-537.

31. Torres-Ruiz RA, Jürgens G: Mutations in the ***FASS*** gene uncouple pattern formation and morphogenesis in ***Arabidopsis*** development. *Development* 1994, 120:2967-2978.

32. Jürgens G, Mayer U: ***Arabidopsis***. In *EMBRYOS: Colour Atlas of Development*. Edited by Bard JBL. London: Wolfe Publishing; 1994:7-21.

33. Natesh S, Rau MA: The embryo. *Embryology of Angiosperms*. Edited by Johri BM. Berlin: Springer-Verlag; 1984.

34. •• Hemerly AS, Ferreira PC, Van Montagu M, Engler G, Inze D: Cell division events are essential for embryo patterning and morphogenesis: studies on dominant-negative cdc2aAt mutants of ***Arabidopsis***. *Plant J* 2000, 23:123-130.
Inhibition of cell division in embryos was achieved using a dominant negative allele of the cdc2a cyclin-dependent kinase. This inhibition was found to cause abnormalities in an apical–basal axis of the embryo, but not in radial patterning. This work shows that cell division is essential for certain aspects of embryonic development.

35. • Grbic V, Bleecker AB: Axillary meristem development in ***Arabidopsis thaliana***. *Plant J* 2000, 21:215-223.
A detailed analysis of the morphology and gene expression of axillary meristem development. Axillary meristem formation occurs in two waves: an acropetal wave during vegetative development and a basipetal wave during reproductive development. Moreover, axillary meristem formation is associated with an increase in *STM* expression.

36. •• Donnelly PM, Bonetta D, Tsukaya H, Dengler RE, Dengler NG: Cell cycling and cell enlargement in developing leaves of ***Arabidopsis***. *Dev Biol* 1999, 215:407-419.
The authors performed a detailed analysis, using a GUS (β-glucuronidase) reporter gene, of cell cycling and cell enlargement during leaf morphogenesis in *Arabidopsis*. The analysis reveals complex patterns of cell cycling, with local events such as stomata formation superimposed on global patterns of proliferation.

37. Glover BJ: Differentiation in plant epidermal cells. *J Exp Botany* 2000, 51:497-505.

38. Szymkowiak E: Is the extent of the proliferation of component cell lineages critical during organ morphogenesis? *Sem Cell Dev Biol* 1996, 7:849-856.

39. Smith LG, Hake S, Sylvester AW: The ***tangled-1*** mutation alters cell division orientations throughout maize leaf development without altering leaf shape. *Development* 1996, 122:481-489.

40. Hemerly A, Engler JD, Bergounioux C, Van Montagu M, Engler G, Inze D, Ferreira P: Dominant-negative mutants of the cdc2 kinase uncouple cell-division from iterative plant development. *EMBO J* 1995, 14:3925-3936.

41. •• Cockcroft CE, Den Boer BG, Healy JM, Murray JA: Cyclin D control of growth rate in plants. *Nature* 2000, 405:575-579.
Faster growth of tobacco was engineered by overexpression of the *Arabidopsis* cyclin CycD2, and was shown to correlate with shorter cell cycles and a significantly increased population of dividing cells in the meristem. Faster overall plant growth, therefore, results from increased cell division, suggesting that cell division rate in meristems may be the main mechanism by which plant growth rate is controlled.

42. •• Mizukami Y, Fischer RL: Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. *Proc Natl Acad Sci USA* 2000, 97:942-947.
Constitutive expression of *ANT* enlarges embryonic and all shoot organs without changing morphology by increasing the cell number. Moreover, expression of *ANT* in fully differentiated organs results in neoplastic formation of callus. *ANT* may regulate cell proliferation and therefore be involved in maintaining meristematic competence.

43. Van Den Berg C, Willemsen V, Hage W, Weisbeek P, Scheres B: Cell fate in the ***Arabidopsis*** root meristem determined by directional signalling. *Nature* 1995, 378:62-65.

44. Van Den Berg C, Willemsen V, Hendriks G, Weisbeek P, Scheres B: Short-range control of cell differentiation in the ***Arabidopsis*** root meristem. *Nature* 1997, 390:287-289.

45. McKibbin RS, Halford NG, Francis D: Expression of fission yeast cdc25 alters the frequency of lateral root formation in transgenic tobacco. *Plant Mol Biol* 1998, 36:601-612.

46. Doerner P, Jørgensen J-E, You R, Steppuhn J, Lamb C: Control of root growth and development by cyclin expression. *Nature* 1996, 380:520-523.

47. De Veylder L, De Almeida Engler J, Burssens S, Manevski A, Lescure B, Van Montagu M, Engler G, Inze D: A new D-type cyclin of ***Arabidopsis thaliana*** expressed during lateral root primordia formation. *Planta* 1999, 208:453-462.

48. • Mews M, Sek FJ, Volkmann D, John PCL: Immunodetection of four mitotic cyclins and the Cdc2a protein kinase in maize root: their distribution in cell development and dedifferentiation. *Protoplasma* 2000, 212:236-249.
The first detailed analysis of the differential abundance of cyclin proteins in any plant tissue. The results suggest that in maize roots specific A- and B-type cyclins may play specific roles in different cell cycle transitions and in the switch from proliferation to differentiation.

49. Meyerowitz EM: Genetic control of cell division patterns in developing plants. *Cell* 1997, 88:299-308.

50. Sillje HH, Takahashi K, Tanaka K, Van Houwe G, Nigg EA: Mammalian homologues of the plant ***Tousled*** gene code for cell-cycle-regulated kinases with maximal activities linked to ongoing DNA replication. *EMBO J* 1999, 18:5691-5702.

51. Meijer M, Murray JAH: The role and regulation of D-type cyclins in the plant cell cycle. *Plant Mol Biol* 2000, 43:621-633.

52. Francis D: Cell size and organ development in higher plants. In *Plant Cell Cycle*. Edited by Francis D, Dudits D, Inze D. London: Portland Press; 1998:187-206.

53. Sekine M, Ito M, Uemukai K, Maeda Y, Nakagami H, Shinmyo A: Isolation and characterization of the E2F-like gene in plants. *FEBS Lett* 1999, 460:117-122.

54. Yamaguchi M, Fabian T, Sauter M, Bhalerao RP, Schrader J, Sandberg G, Umeda M, Uchimiya H: Activation of CDK-activating kinase is dependent on interaction with H-type cyclins in plants. *Plant J* 2000, 24:11-20.