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
cssS2 cell cycle switch S2
cdc25 cell-division cycle protein 25
CDK cyclin-dependent kinase
CycD D-type cyclin
ICK1 interactor of cdc2 kinase 1
pRb retinoblastoma protein
rml1 root meristemless1
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 (G1 and G2) in the sequence G1→S→G2→M [6].

In all eukaryotes, cell cycle regulation depends on cyclin-dependent kinases (CDKs) complexed with their associated cyclins at both the G1→S phase and the G2→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 (G0) [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 G1→S and G2→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 G1, 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 G1 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 G2→M transition, cyclinB–CDK complexes are initially inactive because of inhibitory phosphorylation carried out by the weel 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
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_ (rm1) 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 G1 is caused by the loss of γ-glutamylcysteine synthase [28•]. _rm1_ 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

Figure 1

Model of key checkpoints during cell cycle progression in plants. **(a)** Activation of G1 progression and G1→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 G1, 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)** G2→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••].
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).
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 tossed, 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


An excellent and comprehensive review of the control of the plant cell cycle.

14. CycD3 of Arabidopsis is shown to be induced by the phytomorph 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 G1 → S transition.
24. 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.
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