

# Control of plant growth and development through manipulation of cell-cycle genes

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The plant embryo is a relatively simple structure consisting of a primordial shoot and root, whose development is frozen in the form of a seed. Most development of the mature plant takes place post-embryonically, and is the consequence of cell division and organogenesis in small regions known as meristems, which originate in the embryonic shoot and root apices. Significant recent progress has been made in understanding the mechanisms that control the plant cell cycle at a molecular level, and the first attempts have been made to control plant growth through modulation of cell-cycle genes. These results suggest that there is significant potential to control plant growth and architecture through manipulation of cell division rates. However, a full realisation of the promise of such strategies will probably require a much greater understanding of cell division control and how its upstream regulation is co-ordinated by spatial relationships between cells and by environmental signals.

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## Abbreviations

**CDK** cyclin-dependent kinase  
**FZR** *fizzy-related*  
**pRb** retinoblastoma protein  
**SAM** shoot apical meristem

## Introduction

Multicellular organisms and their constituent organs are formed by a combination of cell growth, proliferation, differentiation and programmed cell death. Three key differences exist between plants and animals, which must be considered when analysing the role of cell division in developmental processes.

The first is that, in contrast to animals, the organs of the mature plant are not formed in miniature in the embryo. In fact, most of what we see of plants is derived from post-embryonic development and originates in the activities of small groups of cells called meristems. The most important of these are the shoot apical meristem (SAM) and root apical meristem, located at the primary growing points of shoots and roots, respectively. Meristems remain active throughout the life cycle of the plant and are responsible for the formation of leaves, stem, flowers, roots and other tissues.

Secondly, plant cells are surrounded by rigid cell walls and are interconnected by cytoplasmic strands through

controlled channels known as plasmodesmata. The rigid attachment of plant cells to each other is necessary to maintain plant shape and rigidity, but means that relationships between adjacent cells are fixed, and cell movement and migration is not possible. Cells are often arranged in sheets or files, and division must therefore be coordinated in some way to ensure that neighbouring parts of the same structure increase in size at the appropriate rate. Very little is understood about the molecular basis of these intercellular controls.

Finally, unlike animals, whose responses to their environment are normally physiological, plants often respond to their environment by changes in the pattern or rate of development. Because plant development is intimately connected with cell division, links between the external environment and the control of the cell cycle are to be anticipated.

Clearly, therefore, cell division is fundamental to plant development, and is an interesting if challenging target for attempts to modify growth or plant form. Provided cell division can be controlled in such a way that it is correctly integrated with plant development, manipulating when and where cell division occurs opens a number of opportunities to change plant architecture and agronomically important traits, such as growth rate and ultimately yield.

For this to be achieved, it is necessary to understand how cell-cycle factors regulate progression through the plant cell cycle, how gene networks contribute to the formation of three-dimensional organ structures, and what makes cells exit the cell cycle to differentiate into the many cell types that are present in plants [1•]. Finally, we should understand how cell growth and division are connected. In this review, we will focus on plants with modulated levels of cell-cycle components and how this affects plant development. We do not cover the genetic control of cell division, but refer the reader to excellent recent reviews [1•,2,3].

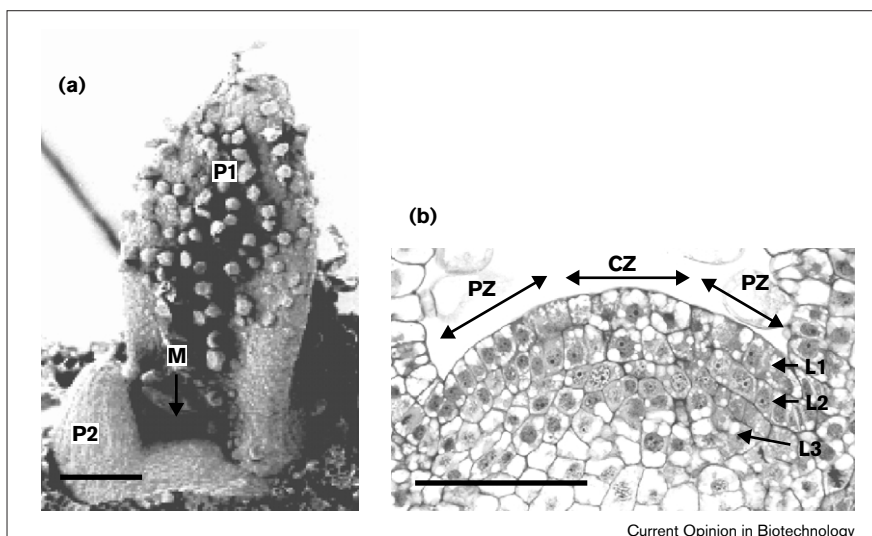
## Spatial organisation of cell division

Meristems are the growing points of the plant and have two basic functions: maintaining a self-perpetuating population of stem cells; and providing the cells that can be set aside to develop into new organs — leaves, stem or flowers [4••] (Figure 1a).

If we take the SAM as an example, we see that it contains a small group of (generally) slow cycling stem cells at the centre of the apex, which are surrounded by a larger group of faster cycling cells at the periphery (Figure 1b). Within this peripheral zone, new organ primordia are initiated by outgrowth from the meristem surface, and these will

**Figure 1**

Shoot apical meristem (SAM) organisation. **(a)** Scanning electron micrograph of a tobacco SAM. Dividing cells originate from the meristem (M), which is seen as a slight dome between leaf primordia, and then get displaced laterally and incorporated into leaf primordia (P2). Older leaf primordia (P1) contain few dividing cells and the final stages of leaf development are marked mainly by cell expansion. Bar = 100  $\mu\text{m}$ . **(b)** Section through a tobacco SAM. Note the layered structure of the dome. CZ, central zone of slower cycling stem cells; L1, epidermal layer; L2, sub-epidermal layer; L3, corpus or layer 3; PZ, peripheral zone of faster cycling cells. Bar = 100  $\mu\text{m}$ .



develop into organs such as leaves or flowers. A second level of organisation is that the outer two cell layers (L1 and L2) of the meristem dome are clonally distinct from the inner (L3) layer (Figure 1b). An active meristem therefore has to maintain cells with different cell cycle times within the layered configuration, as well as provide sufficient cells both to maintain the meristem and to form organs [2,4\*\*].

If we want to start to think about manipulating cell division, we have to know where cell division occurs in the meristem and what affects meristem activity. Using *in situ* hybridisation with probes for genes whose RNA is only present at specific cell-cycle phases, or incorporation of a label into replicating DNA, it was found that dividing cells are scattered throughout the inflorescence SAM of snapdragon (*Antirrhinum*) and the vegetative SAM of pea, and that neighboring cells are not synchronised in their cell division [4\*\*,5]. Furthermore, cells that are cycling slowly or are temporarily not cycling are scattered throughout all regions of the meristem [6], so the SAM is a complex mosaic of cells cycling at different rates and at different stages of the cell cycle [4\*\*].

Important when considering the control of cell division by transgene expression is the accumulating evidence that environmental fluctuations affect the cell division cycle by unknown signalling routes and change both the length of the cell cycle and the proportion of cells in the meristem that are rapidly cycling [7]. In the case of exposure of the grass *Dactylis* to increased CO<sub>2</sub> levels, a detailed analysis showed that the latter change is the most significant, and significantly that the increased rate of cell proliferation results in faster growth [8]. Temperature also has dramatic effects on both plant growth and cell cycle, affecting mainly the length of the G1 phase [9]. Manipulation of cell

division may therefore allow control of aspects of plant environmental responses.

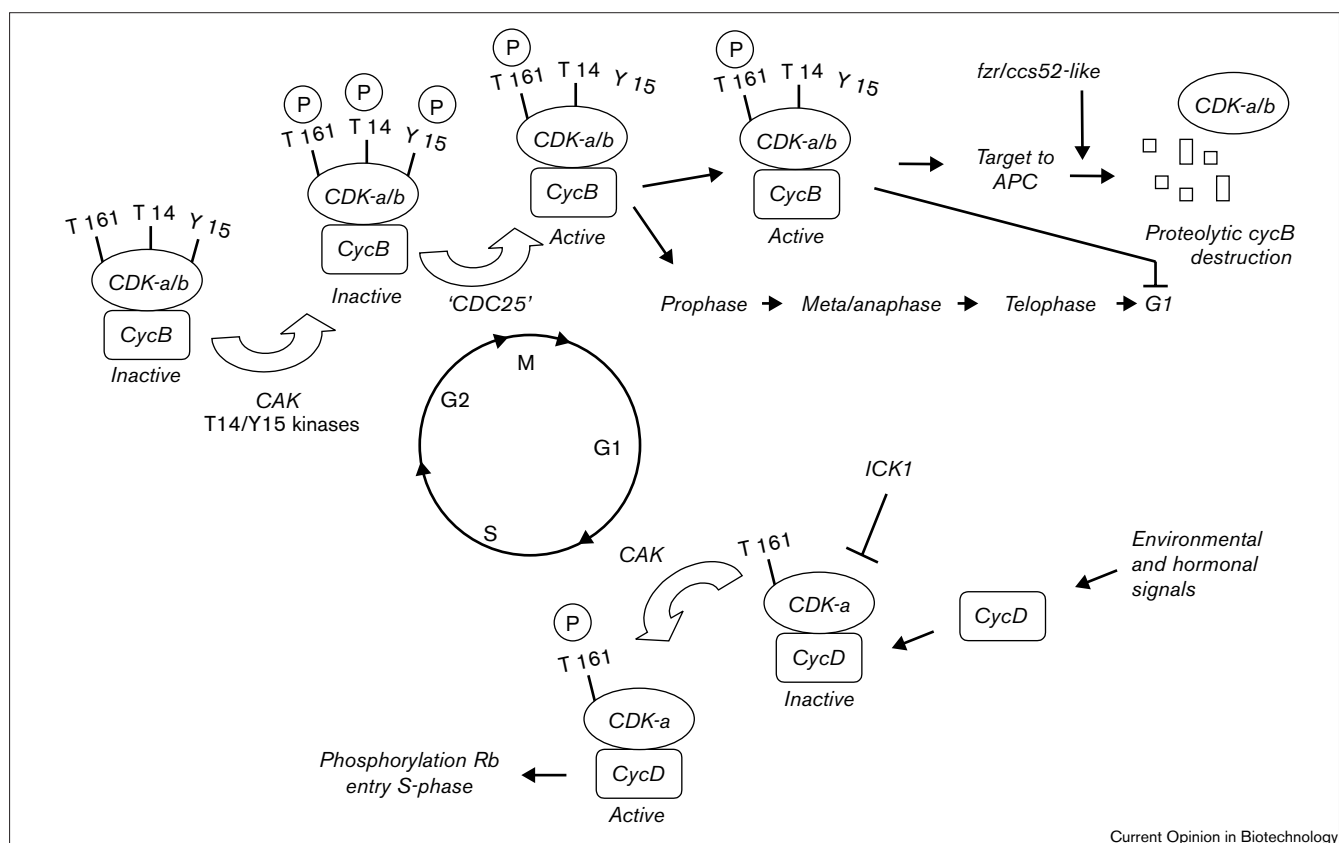
### The cell cycle: cyclin-dependent kinases and cyclins

The cell cycle consists of four phases in the order G1→S→G2→M, which provides for the temporal separation of the processes of DNA synthesis (S phase) and mitosis (M phase).

In yeasts, mammals and plants, progression through the cell cycle is driven by periodic peaks in cyclin-dependent kinase (CDK) activity [10,11\*\*]. CDKs consist of a catalytic subunit (the CDK itself), which is inactive without an appropriate regulatory partner (cyclin) with which to dimerize. A primary mechanism of CDK regulation is therefore through regulated transcription and proteolysis of cyclins, and CDKs and cyclins are primary candidates for cell-cycle manipulation.

Yeasts possess only one main type of CDK (*cdc2* in *S. pombe* and *CDC28* in *S. cerevisiae*) that is responsible for all cell-cycle transitions. The yeast CDK contains the sequence PSTAIRE (amino acid single letter code) in its cyclin interaction domain, and CDKs with this sequence that can complement yeast *cdc28* mutants are found in all eukaryotes [11\*\*,12]. In plants, three classes of CDKs have been identified. The CDK-a class consists of PSTAIRE-containing CDKs, which are widely expressed in many plant tissues [11\*\*]. Although present throughout all cell-cycle phases, their kinase activity peaks at the G1→S and G2→M phase transitions. The CDK-b class contains two sub-groups with either PPTALRE or PPTTLRE motifs [13,14], and are unique amongst CDKs in exhibiting cell-cycle regulation of gene expression during S, G2 and M phases [11\*\*,13]. The third class includes

Figure 2



Key checkpoints during cell-cycle progression in plants. CDK activities peak during G1→S and G2→M transitions. External and intrinsic signals result in the rising levels of D-type (*CycD*) cyclins, which associate with *CDK-a*. Activation of the complex on T161 by *CDK* activating kinase (CAK) is required, but it is unknown if this is a regulatory step. The *CDK* inhibitor ICK1 inhibits kinase activity of the complex [38\*]. In late G1, rising levels of *CycD*–*CDK-a* kinase activity lead to phosphorylation of retinoblastoma (Rb). Phosphorylation renders Rb inactive, the E2F transcription factor is no longer repressed and S-phase genes are transcribed (see text). Cells that approach

M phase contain inactive *CDK*–*CycB* complexes, requiring both CAK activation and removal of inhibitory phosphates on T14 and Y15 by *CDC25*. The active complex triggers entry into prophase. A further control operates between telophase and G1, which requires the destruction of mitotic *CDK* activity by anaphase-promoting complex (APC). The targeting of specific components may involve *ccs52*, a gene related to *Drosophila* FZR [55\*\*]. This model is mainly based on the mammalian cell cycle, but most factors have been identified in plants except the *cdc25* phosphatase.

other *CDK*-like genes, including *CDK* activating kinases (CAKs; see below).

In mammalian cells, the crucial target of G1→S *CDK* activity is the retinoblastoma protein (pRb) [15]. During G1 phase, pRb inhibits expression of E2F-regulated promoters by directly binding to E2Fs and thereby blocking transcription, as well as by recruiting histone deacetylase, which leads to a local remodelling of chromatin. The phosphorylation of pRb by *CDK*s results in its dissociation from E2F, and allows activation of the various target genes of E2F needed for S-phase entry [16]. During most of G1, *CDK* activity depends on a class of cyclins known as D-type cyclins, whose transcription is under direct regulation by growth factors in the extracellular medium, and this creates a key link between such external growth-promoting signals and cell-cycle control.

Although this pathway is not conserved in yeast, it has recently become clear that it is present in plants. cDNAs encoding D-type cyclins (*CycD*) [17–19], pRb [20,21] and E2F proteins [22\*,23\*] have all been cloned and probably function in a very similar way to their mammalian counterparts [24\*]. As in animals, plant D-type cyclins are also involved in responses to extracellular signals, such as plant hormones and nutrient status [14,25\*\*]. Furthermore, the finding that accumulation of maize pRb is correlated with cell differentiation makes it probable that analogous roles exist for the pRb pathway in control of the balance between cellular proliferation and differentiation in both animals and plants [16,24\*].

In addition to D-type cyclins (*CycD*), plants also contain A- and B-type cyclins, which are expressed during S→M or G2 and M phases, respectively, as in most other higher

eukaryotes [19]. In plants, these probably associate with CDK-a and/or CDK-b to create G2→M-associated kinase activities whose most probable targets are nuclear and cytoplasmic proteins the phosphorylation of which is required for entry into mitosis [26].

An important mechanism by which CDK activity is regulated during the cell cycle is by the periodic synthesis and destruction of cyclins. Cyclins operating during the G2→M transition contain a defined 'destruction-box motif', which targets them for degradation by ubiquitin-mediated proteolysis via the anaphase-promoting complex (APC). Substrate specificity of the APC is determined by its association with further proteins, including FZR (*fizzy-related* from *Drosophila*, of which more later). The D-type cyclins operating during the G1→S transition have so-called PEST sequences, which makes them unstable [27].

### Further modes of CDK regulation

In addition to cyclin binding, both phosphorylation of the CDK subunit and the association of inhibitory proteins are important additional mechanisms of regulation [28,29].

All CDKs require phosphorylation of a conserved threonine residue (usually T160 or 161) for activity, in order to displace the so-called T-loop and open up the ATP-binding site. This is carried out by a CDK activating kinase, of which two types have been identified in plants [30,31\*].

In addition, the activity of CDKs is blocked by inhibitory phosphorylation on threonine (T14) and/or tyrosine (Y15) residues near their amino terminus, which in animals and yeast is carried out by the *wee1* and *mik1* kinases [32,33]. Activation requires dephosphorylation by the *cdc25* phosphatase [34], which triggers mitosis in fission yeast [35]. A

homologue of *wee1* has recently been identified in maize [36\*], but no plant *cdc25* genes have been reported to date.

CDK inhibitor proteins (CKIs) provide a further level of regulation. They bind to the CDK, the cyclin or the CDK–cyclin complex and inhibit their association or activity [37]. Four different types of CKI have been reported in plants, but details have only been published on the ICK1 inhibitor from *Arabidopsis*, which binds the D-type cyclin CycD3 and inhibits CDK activity [38\*].

### Manipulation of the plant cell cycle

From the discussion above, it should be clear that manipulation of cell-cycle genes could have a number of effects if cells are accelerated or inhibited in a specific cell-cycle stage. One possibility is that the shortening of one cell-cycle phase leads to compensatory increases in other phases, so that the overall cell-cycle length is unaffected. This has generally been observed in mammalian cells with accelerated G1 phases caused by overexpression of the G1 cyclins D or E [39,40]. In other situations, however, for example, in yeast with increased G1 cyclin activity [41] or defective in the mitotic CDK inhibitory kinase *wee1*, smaller cells result from precocious progress through the division cycle. So changes in cell size and possibly cell-cycle length might be expected. The discussion above also makes clear, however, both the importance of the spatial organisation of cell division and the inter-relationship of cell division and differentiation. Wider changes in the developmental pattern might therefore be observed either due to ectopic divisions or changes in differentiation. Finally, cell differentiation in plants can be accompanied by an increase in cell ploidy level resulting from alteration of the cell cycle might affect the switch from mitotic cycles to endoreduplication cycles (e.g. in the formation of seed endosperm).

**Table 1**

#### Phenotypes of modulation of cell-cycle components at the whole-plant level.

Cell-cycle gene	Modulation	Phenotype (transgenic plant)	Reference
<i>cycB1(cyc1At)</i>	Overexpression in root from <i>cdc2a</i> promoter	Enhanced root growth ( <i>Arabidopsis</i> )	[42]
<i>cycD3(At)</i>	Constitutive expression	Curly leaves, disorganised meristems, and delayed senescence ( <i>Arabidopsis</i> )	[25**]
<i>CDK-a(At)</i>	Constitutive expression	No effect ( <i>Arabidopsis</i> )	[43]
<i>CDK-a(At)</i> <i>N147A223</i>	Constitutive expression	Lethal ( <i>Arabidopsis</i> )	[43]
<i>CDK-a(At)</i> <i>N147A223</i>	Constitutive expression	Normal stature, and reduced number of larger cells (tobacco)	[43]
<i>CDK-b(At)</i>	Inducible downregulation	Dark grown seedlings have short hypocotyls and open cotyledons ( <i>Arabidopsis</i> )	[45*]
<i>cdc25(yeast)</i>	Constitutive expression (low level)	Precocious flowering, aberrant leaves and flowers, and smaller cells in side roots (tobacco)	[46]
<i>cdc25(yeast)</i>	Inducible expression	Increased frequency of lateral root formation and reduced mitotic cell size (tobacco)	[47*]
<i>ccs52(fizzy-related) (Ms)</i>	Constitutive downregulation	Decreased ploidy level and cell size ( <i>Medicago truncatula</i> )	[55**]

*At*, *Arabidopsis thaliana*; *Ms*, *Medicago sativa*.

### Cyclins

Plant cyclins are divided into mitotic cyclins (CycA and CycB) and G1 cyclins (CycD cyclins), with further conserved subgroups within these classes [19]. Upon expression in roots of the *Arabidopsis* mitotic cyclin CycB1 (originally called *cyc1;At*) under control of the relatively weak but widely expressed *cdc2a* (CDK-a) promoter, root growth rate was increased in *Arabidopsis* [42] (Table 1). This increase was mainly a result of an increase in cell number rather than cell size but the mechanism by which this occurs by CycB1 is unclear. Following induction of lateral roots with auxin, plants overexpressing the mitotic cyclin developed an enlarged root system. It is unclear what mechanism underlies the enhanced growth, as no CDK activity nor cell-cycle times were reported. The conclusion from this work is that mitotic cyclins, and control at the G2→M phase boundary, appear to be limiting for root growth in *Arabidopsis*.

In contrast to the expression of *CycB1* being specific to the cell-cycle stage, the D-type cyclin *CycD3* of *Arabidopsis* is induced rapidly by cytokinin during the G1 phase of cells re-entering the cell cycle. Constitutive expression of this cyclin leads to extensive leaf curling, disorganised meristems and a delayed senescence in *Arabidopsis* [25••]. The development of the plant is retarded, although flowering occurs with a wild-type leaf number (C Riou-Khamlichi, BGW den Boer, JAH Murray, unpublished data). Leaves from *Arabidopsis* plants expressing high levels *CycD3* form callus in culture in the absence of cytokinin, which also become green. Mechanistically speaking, these results suggest that cytokinin activates *Arabidopsis* cell division in dedifferentiating leaf cells through induction of *CycD3*. In contrast, *CycD2* is induced earlier in G1 phase than *CycD3* and is sucrose responsive in *Arabidopsis* (C Riou-Kahmlichi, M Menges, S Healy, JAH Murray, unpublished data).

### CDKs

Several approaches have been used to change CDK-a activity in plants. Constitutive expression of CDK-a in *Arabidopsis* or tobacco leads to a less than twofold increase in CDK activity as assayed on the substrate histone H1 [43]. The transgenic plants showed no obvious phenotype, except for the tendency of some *Arabidopsis* plants to lose apical dominance (which results in the outgrowth of side shoots). Clearly, CDK-a is not normally a limiting factor for plant cell division.

Post-translational modification of CDKs by phosphorylation on conserved residues has also been attempted. Phosphorylation of T14 and Y15 leads to inhibition of the kinase function in yeast and animals, and Y15 has been found in all reported plant CDK-a homologues. Phosphorylation of Y15 may be part of a control mechanism in plants, as tobacco pith parenchyma or suspension-cultured cells arrested in G2 by the absence

of the plant hormone cytokinin contain CDK-complexes with reduced kinase activity and high phosphotyrosine content. The CDK is re-activated *in vitro* by treatment with the yeast *cdc25* phosphatase, which is highly specific for Y15 of CDKs [44].

Constitutive expression of a CDK-a containing T14 and Y15 replaced by non-phosphorylatable amino acids had no effect, however, despite high levels of expression of the mutant protein [43], although this would be predicted to give a dominant phenotype due to lack of mitotic regulation. This result suggests that other factors may be rate limiting for mitotic progression *in planta*.

The only mutant allele of CDK-a that had drastic effects upon development involved substitution of D147, which eliminates CDK activity because this residue is required for transfer of the  $\gamma$ -phosphate group of ATP to substrates. Although no *Arabidopsis* plants could be obtained after constitutive expression of this allele, some tobacco lines could be recovered that showed about a twofold reduction in overall histone H1 kinase activity [43]. Intriguingly, these mutant tobacco plants had a normal structure, but a reduced number of cells that were all larger compared to control plants [43]. This may be interpreted as an uncoupling of cell division from cell growth, due to inhibition of cell division. Apparently, changing the number of cells in a plant does not affect its overall appearance, indicating that cells correctly interpret positional information despite their reduced number, perhaps because architectural control operates more through cell growth than cell division. It would also be interesting to know whether ploidy levels have been changed in these plants, as endoreduplication is often associated with increased cell size.

CDK-b (PPTALRE/PPTTLRE) CDKs are unique to plants and it is therefore interesting to understand their roles. Downregulation of the CDK-b (*cdc2b*) gene of *Arabidopsis* was achieved by using an inducible antisense construct [45•]. After induction, endogenous *cdc2b* mRNA levels declined and seedlings showed dose-dependent phenotypes. When germinated in the dark, seedlings expressing the antisense *cdc2b* transgene had a short hypocotyl and open cotyledons in contrast to the wild-type plants, which have long hypocotyls and closed cotyledons. This phenotype was shown to be caused by a reduction in length of the hypocotyl cells and an expansion of epidermal cotyledon cells. Notably, down regulation of *cdc2b* had no effect on the endoreduplication of hypocotyl cells, indicating that this is not a regulating factor for this process.

### Post-translational triggering of CDK activity

Although no direct homolog of *cdc25* phosphatase has been identified in plants, the fission yeast *cdc25* has been expressed in tobacco [46,47•]. Overexpression in fission yeast resulted in a decreased cell size at mitosis [48].

Tobacco plants constitutively expressing fission yeast *cdc25* displayed precocious flowering, aberrant leaves and flowers, and cell size at division in lateral roots was smaller compared to control plants [46]. To confirm directly the action of *cdc25*, the gene was expressed under the control of an inducible promoter [47\*]. In roots, a 2–3.5-fold greater frequency of lateral root primordia containing smaller mitotic cells was observed. These results are additional indications that CDK activity in plants is regulated in part by dephosphorylation via a *cdc25*-like phosphatase, which might be under control of the plant hormone cytokinin [44]. It is not clear, however, whether the *cdc25* phosphatase could also affect CDK activity at the G1→S transition, as in mammals there are three *CDC25* genes with specific functions at G1→S and G2→M.

### Mitotic inhibitor inducing endoreduplication

Reduplication of the genome without mitosis (endoreduplication) is a common feature of plant development. Hypocotyl cells [49\*], trichomes [50], leaf pavement cells [51] and developing endosperm of seeds [52] are cells and tissues undergoing endoreduplication. In *Drosophila*, the switch from a normal cell cycle (G1→S→G2→M) to an endoreduplication cycle (G1→S→G2) requires turning off G2→M cyclins (cyclins A and B) and periodic expression of the G1→S-phase-specific cyclin E [53]. Similarly, in maize endosperm it has been shown that endoreduplication involves inhibition of M-phase-promoting factor (MPF; p34<sup>cdc2</sup>-cycB complex) and induction of S-phase-related protein kinases [54].

A first successful attempt to modulate endoreduplication in plants using cell-cycle components was reported recently. Constitutive expression of *acs52* (a plant homologue of FZR/hCDH1) cDNA in the antisense orientation in *M. truncatula* showed a direct correlation between reduction in *acs52* transcript levels and a decrease in polyploidy and cell size [55\*\*]. The rationale behind this is that *acs52* shows high homology to *Drosophila* FZR. FZR from flies has been shown to promote the degradation of specific mitotic cyclins in G1 and the exit from the cell cycle [56]. It is not clear whether downregulation of *acs52* is accompanied by an accumulation of mitotic cyclins. Nevertheless, this work suggests that a target of *acs52*-directed proteolysis is normally an inhibitor of endoreduplication.

### Conclusions

Recent work in the field of plant cell division has identified many of the components of the cell-cycle machinery, as well as factors that can influence its functioning. In order for cell-cycle components to be used widely to manipulate plant growth and development, further focus is probably required on several areas: firstly, how cell growth is coordinated with cell division [57\*] and development [58\*]; secondly, how environmental signals feed into the cell-cycle machinery; and finally, from a technical point of

view, how gene activation or inactivation can be better targeted to specific cells or cell compartments. Nevertheless, initial experiments indicate that cell-cycle genes are interesting targets for the modification of plant growth, architecture and yield.

### Acknowledgements

We apologize to those whose work was not mentioned or discussed fully due to space constraints.

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