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Review

The developmental context of cell-cycle control in plants

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Abstract

Plant growth is characterised both by continued growth and organogenesis throughout development, as well as by environmental influences on the rate and pattern of these processes. This necessitates a close relationship between cell cycle control, differentiation and development that can be readily observed and studied. The sequencing of the Arabidopsis genome has revealed the full complexity of cell cycle regulators in plants, creating a challenge to understand how these genes control plant growth and differentiation, and how they are integrated with intrinsic and external signals. Here, we review the control of the cell cycle and examine how it is integrated with proliferative activity within meristems, and during the differentiation processes leading to leaf and lateral root formation.
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Keywords: Cell division; Arabidopsis; Cyclins; CDK; Differentiation; Leaf development; Root development

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1. Introduction—the developmental context of plant cell cycling

Although as we shall see, the overall control of the cell cycle is broadly similar between plants and other eukaryotic organisms, the pattern of plant development dictates that it is organised spatially and temporally in radically different ways.

Plant development differs from that of animals in four main aspects. First, whereas animal development produces a mature embryo that already possesses the major organ systems of the post-embryonic organism, plant embryogenesis is mainly concerned with the production of groups of stem cells known as meristems at the apical and distal ends of the mature embryo which will then continue during adult growth to produce the organs that build the adult plant. Granted these stem cells are associated with an embryonic root and shoot that already possesses one or two seed leaves (cotyledons), but nevertheless plant post-embryonic growth involves not only increases in organism size but also the production of almost all organs. This leads us to the second major difference between plants and animals, which is that plant post-embryonic growth is associated with an organogenic continuum in which

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stem cells continuously produce new organs (leaves, roots, flowers) through de novo initiation, in contrast to the temporal restriction of animal organogenesis to the specific developmental stages of embryogenesis and pupation.

The third major difference is related to the sessile nature of plants and the importance of developmental responses to the environment in which they find themselves. Factors such as light, temperature and nutrient availability will impact on both the rate and, in some cases, the pattern of plant growth. Hence, such influences will impact on the cell cycle because of the importance of cell production in both growth and organogenic processes.

Finally, the nature of plant cells surrounded by a rigid cell wall imposes restrictions on the types of cell behaviour involved in developmental processes, in particular precluding cell migration as a mechanism for establishing pattern.

In broad terms, these represent the developmental context in which cell division must be placed in plants. We first explore the major differences between what is known of cell cycle control in plants and animals, and then examine aspects of cell division within meristems and during leaf and lateral root organogenesis.

2. The cellular perspective of the mitotic plant cell cycle

Plants share with all other eukaryotes the basic phases of the cell cycle (Table 1), and the control of transitions by cyclin-dependent kinase (CDK) complexes. Altogether, Arabidopsis has 49 cyclins in 10 classes [1], but cyclins with primary cell cycle roles are represented by the classes of A-, B-, and D-type cyclins, no plant cyclin E homologue has been identified. Plants also differ in their CDK repertoire compared to animals or yeasts, containing not only the archetypal *cdc2* (CDK1) homologue with the canonical PSTAIRE sequence (single-letter amino acid code) in their cyclin-binding domain and known in plants as CDKA, but also a plant-specific group of CDKs known as CDKB. Plants, therefore, lack equivalents of the cyclin D-binding CDK4 and CDK6, and plant D-type cyclins appear to bind primarily CDKA [2] and in some cases CDKB [3]. Given the constitutive nature of CDKA expression and activity, and the restricted expression of *CDKB* genes to S-phase and later, it is assumed that CDKA provides the major G1 kinase activity, and that this is joined in G2/M by additional CDKB activity.

Control of plant mitotic cycles is regulated at the G1/S transition by D-type cyclins (CYCD), represented in Arabidopsis by 10 genes in seven sub-classes. Plant CYCDs have low homology to vertebrate D-type cyclins, and not all contain the LxCxE Rb-binding domain [4]. Expression of several CYCDs is regulated by nutrient availability and hormones (reviewed [5]). When analysed in synchronous cell cultures, most CYCDs increase during cell cycle re-entry, but during the subsequent cell cycles expression remains relatively constant, consistent with a primary role in responding to external signals [6]. However, two members of the CYCD family show cyclical expression: CYCD5;1 has peak expression in G1 and CYCD4;1 in late G1/S-phase [7], suggesting possible roles as functional equivalents of cyclin E. Certainly overexpression of CYCDs is sufficient to drive cells into S-phase, resulting in a shortened G1-phase and dramatic effects on leaf development (see below).

Table 1
List of Arabidopsis homologues of key cell cycle components and the phase of the cell cycle they are thought to play a role in

Homologue	Cell cycle phase of activity	Arabidopsis proteins
Cyclins: D-type	G1/S transition (and G2/M)	CycD1;1 CycD2;1 CycD3;1; -3;2; -3;3 CycD4;1; -4;2 CycD5;1 CycD6;1 CycD7;1
Cyclins: A-type	G1/S; S-phase	CycA1;1; -1;2 CycA2;1; -2;2; -2;3; -2;4 CycA3;1; -3;2; -3;3; -3;4
Cyclins: B-type	G2/M transition	CycB1;1; -1;2; -1;3; -1;4 CycB2;1; -2;2; -2;3; -2;4 CycB3;1
CDKs	G1/S transition; G2-phase	CDKA;1
Plant specific CDKs	G2/M transition G2-phase; mitosis	CDKB1;1; -1;2 CDKB2;1; -2;2
CAKs		CycH;1/CDKD;1; -2; -3; CDKF;1
KRPs	G1/S transition	KRP1; -2; -3; -4; -5; -6; -7
RBs	G1/S transition	RBR
E2F family	G1/S transition	E2Fa; -b; -c DPa; -b DEL1; -2; -3
WEE1	G2/M transition	WEE1
CDC25	G2/M transition	CDC25
FZR	Metaphase–anaphase	CCS52B

Regulation of CYCD activity also occurs at the level of protein stability and kinase activity. CYCD3;1 is an unstable protein (half-life 7 min) and is targeted for ubiquitin-dependent proteasomal degradation after hyperphosphorylation. During the cell cycle, CYCD3;1 protein levels mirror expression, and several lines of evidence suggest it acts as a transducer of plant hormonal signals into the cell cycle. CYCD2;1 protein is more stable, fluctuating little during the cell cycle, but is regulated at the level of kinase activity [8].

The predominant kinase partner of most CYCDs is CDKA;1, the only Arabidopsis CDK containing the hallmark PSTAIRE motif [4]. Neither expression nor protein abundance fluctuates during the cell cycle, but kinase activity peaks in S-phase and in G2 [9]. While CYCD3;1-associated kinase activity mirrors protein abundance, CYCD2;1-

associated kinase activity is strongly regulated even with constant protein levels during re-entry into the cell cycle. Low CYCD2;1-associated kinase activity is correlated with loss of CDKA;1 interaction, possibly with CYCD2;1 sequestered in a catalytically inactive complex [2].

CDK activity is regulated at additional levels. Arabidopsis has two classes of CDK-activating kinases (CAKs): heterodimeric CDKD/cycH and monomeric CDKF;1, as well as seven CDK inhibitors of the Kip/Cip family (KRPs; [4]), although their homology to mammalian KIP (and indeed to other KRPs in plants) is generally limited to a small functional domain. These KRPs (Kip-related proteins, also known as ICK) show differential interactions with CDKs and cyclins. Most interact with CDKA and CYCDs, and KRPs inhibit CDKA kinase activity (reviewed [5]). Expression patterns of KRPs vary during the cell cycle suggesting different roles [7]. The INK4-type of inhibitor is not found in plants.

The activation of the mitotic cell cycle and entry into S-phase is controlled by a pathway involving activation of E2F activity through phosphorylation of Rb (Retinoblastoma-related protein or RBR) by CYCD–CDKA complexes (Fig. 1). CYCDs containing an LxCxE motif interact with Rb and CDKA–CYCD complexes exhibit kinase activity against Rb (reviewed [10,11]). Only a single Rb homologue has been identified in Arabidopsis [4], with highest RNA expression and RBR-associated kinase activity in proliferative tissues. RBR protein abundance is highest in differentiated tissues.

Phosphorylation of Rb by CDKA–CYCD kinase relieves repression of E2F transcription factors. There are three E2Fs, two DPs and three monomeric E2F-like proteins in Arabidopsis [4]. E2Fa and E2Fb are transcriptional activators, while E2Fc is a repressor similar to mammalian E2F6 although able to interact with Rb (reviewed [11,10]). Expression of E2Fa–c and DP is reasonably constant during the cell cycle, although highest at S-phase, and is associated with cell proliferation and endoreduplication (reviewed [12]). Protein abundance has yet to be determined. Nuclear localisation of E2Fa and E2Fb is promoted by DPa, and that of E2Fc by DPb, possibly due to dimerisation blocking a nuclear export signal. E2Fa and E2Fb preferentially interact with DPa, and E2Fc with DPb in yeast two-hybrid assays, although all three E2Fs, in combination with either DP, can bind consensus E2F-sites. Additionally, E2Fa, E2Fb and E2Fc, together with either DPa or DPb, transactivate gene expression to differing extents [13,14]. The composition of the E2F heterodimers in planta and during the cell cycle has not been determined.

The monomeric E2Fs, known as ELPs [15], E2Fd-f [14], E2Ls [16] or DELs [4], bind E2F-sites as monomers repressing the activity of E2F activators. Repression is Rb-independent as they lack a C-terminal Rb-binding domain [16,14]. DEL2 expression is not strongly regulated during the cell cycle, while DEL1 and DEL3 show expression in S- and G2-phase [14,7].

E2Fs regulate the expression of genes involved in DNA synthesis and replication via interaction with E2F-sites

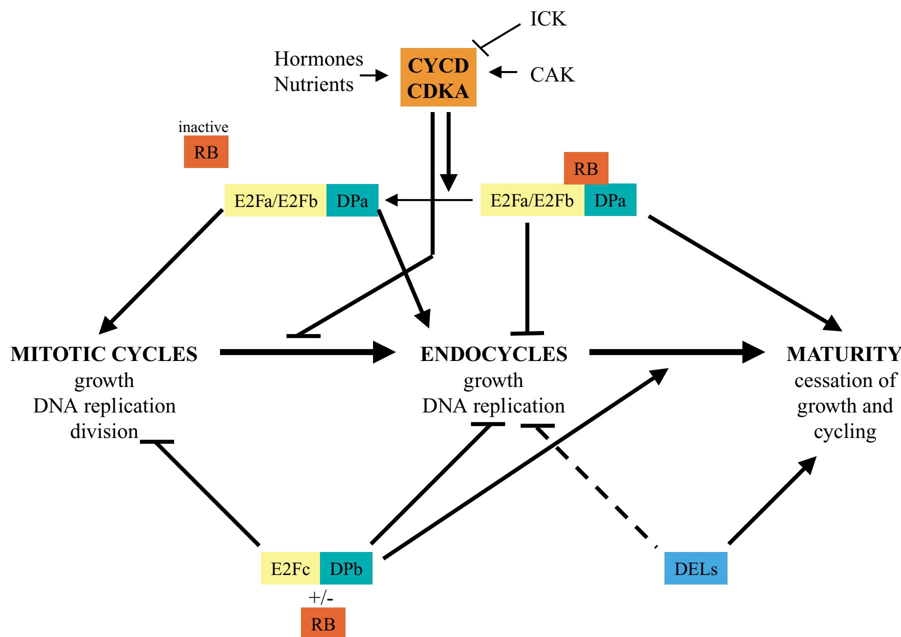

Fig. 1. The relationship of cell cycle activity to developmental progression during the three phases of development of a leaf (mitotic, endocycle, and maturity phases). Within the early leaf primordium, rapid mitotic cycles lead to an increase in cell number, promoted by active CYCD–CDKA complexes. Phosphorylation of RB by CYCD–CDKA complexes (themselves also regulated by CAK and ICK/KRP proteins) activates E2F complexes capable of promoting cell cycling-both mitotic and endocycles. E2Fc is an inhibitory E2F that may promote differentiation. The transition from mitotic cycles to endocycles is accompanied by, and requires, the down-regulation of CYCD activity, and CYCD overexpression inhibits this transition. The endocycling phase is characterised by G–S–G cycles, and is promoted by E2F activity and characterised by lack of expression of normal G1 (CYCD) and G2/M (CYCB) regulators. Final differentiation and maturity is probably promoted by high RB and inhibitory E2F-related proteins (DELs) that switch off E2F responsive genes in an RB-independent manner.

(TTT(C/G)(C/G)CG(C/G)(C/G)) in their promoters. 5765 Arabidopsis genes contain potential E2F-sites in their promoters [17]—over 20% of the genome, including numerous genes involved in DNA synthesis and replication (MCMs, ORC1, CDC6, RNR1), and overexpression studies have identified some of these as potential bone fide targets [18]. Promoter analysis revealed that E2F-sites drive meristematic and S-phase induced expression of these genes, and they were induced by overexpression of E2Fa/DPa. E2Fs also play a role in gene repression outside of S-phase, with E2Fc able to reduce CDC6 expression (reviewed [10,12]). Interestingly, CYCD–CDKA kinase phosphorylates E2Fc leading to ubiquitin-mediated proteasomal degradation [19]. Thus, CYCD kinases may alleviate repression of E2F target genes by phosphorylation of Rb and by targeting E2Fc for degradation. The CDC6 and ORC1 promoters were not bound by DEL3 [20], suggesting differential affinities and a role outside cell division.

Genes with E2F-sites include those involved in signal transduction, metabolism and biogenesis [17], some of which were induced and others repressed by E2Fa/DPa overexpression [18]. Whether these were direct targets of E2Fa/DPa remains unclear. Genes involved in cell wall biogenesis have been found to be direct targets of DEL3, which may play a role in plant growth [20].

A-type cyclin expression is induced sequentially from G1/S through S-phase. The 10 Arabidopsis CYCAs fall into three subclasses [4]. CYCA3 expression begins at the G1/S boundary and precedes that of members of the CYCA1 and CYCA2 subgroups [7]. There are exceptions: alfalfa CYCAs are regulated at the level of kinase activity, not transcription [21]. CYCA3 forms active kinase with CDKA and is required for re-entry into cell division and drives S-phase, suggesting an alternative or additional candidate functionally replacing vertebrate cyclin E [22]. In contrast, CYCA1 and CYCA2 play a role more analogous to vertebrate cyclin A. These are expressed from mid-S-phase of the cell cycle [7], with CYCA2 kinase activity present during S-phase and G2/M [21]. CYCA2 interacts with CDKA and Rb [21]. Additionally, E2Fc is phosphorylated by CYCA–CDKA resulting in ubiquitin-mediated proteasomal degradation [19].

The G2/M transition is controlled primarily by cyclin B. The nine Arabidopsis CYCBs fall into three subclasses, CYCB3s lacking a destruction box [4]. As anticipated, CYCB expression is found only in proliferating tissues, with transcription increasing during G2, and peaking in early mitosis (reviewed [5]). M-phase specific expression is regulated by Myb transcription factors: MybA1 and MybA2 bind the MSA (M-phase specific activator) element activating expression, whereas MybB inhibits it (reviewed [23]). CycB levels are tightly regulated by proteolysis by APC during the metaphase to anaphase transition, a requirement for exit of mitosis (reviewed by Capron et al. [24]), with mitosis impaired by overexpression of non-degradable cycB [25]. CCS52B, a homologue of Drosophila fizzy-related (FZR) and activator of the APC, is expressed in dividing cells during G2/M [7], where it promotes the metaphase–anaphase transition. CYCB is localised to condensing chromatin until anaphase, and CDKA to the preprophase band (a belt-like arrangement of microtubules and actin encircling the future division plane), condensing chromatin, the metaphase spindle, and the phragmoplast (which organises the synthesis of the new cell wall between the daughter cells), suggesting a role in microtubule dynamics (reviewed by Criqui and Genschik [23]).

CYCB interacts with both CDKA and with CDKB [25]. In all plants examined to date, there appear to be at least two different sub-groups called CDKB1 and CDKB2 and [4]. Plant CDKBs appear to be unique in showing strong cell-cycle regulation of expression and of protein abundance. CDKB1 contains a PPTALRE consensus, and expression starts in S-phase with peak expression, protein levels and kinase activity at G2/M. CDKB2 contains a PPTTLRE consensus and expression is confined to G2 and mitosis (reviewed by Dewitte and Murray [5]). Like CDKA, CDKB colocalises to microtubular structures. These kinases may regulate cytoskeleton reorganisation by phosphorylation of microtubule associated proteins (MAPs) and kinesin-related proteins (reviewed by Criqui and Genschik [23]).

CYCB–CDK kinases may be regulated at a number of levels. However, none of the CDK inhibitors tested interacted with CDKB or cycB [26–28]. Wee1 kinase homologues, which add inhibitory phosphates to CDKs have been identified in plants [29,30]. Until recently no homologue of CDC25, the activating phosphatase partner to wee1 had been described in plants. Recently, a homologue with tyrosine-phosphatase activity able to stimulate CDK activity was described [31,32].

3. Endoreduplication

Differentiating plant cells within certain tissues often display endoreduplication, a modified mitotic cycle in which nuclear ploidy increases due to repeated S-phases without intervening mitoses. Endoreduplication occurs after cells have ceased mitotic cycles and endoreduplicated cells do not reenter the mitotic cell cycle, and is thus characteristic of a differentiation phase lying between the mitotic phase and the cessation of all cycling activity (see below).

In Arabidopsis final ploidies attained by certain leaf cell types is 32C, but this is greatly surpassed by *Arum maculatum* endosperm cells, which can reach 24576C. It should, however, be appreciated that not all cells within a tissue necessarily undergo endoreduplication. Endoreduplication is also a characteristic of certain specialised cell types such as trichomes (leaf hairs) and endosperm (found in some seed types and acting as embryonic food reserves) as well as many other cell types. Endoreduplication may be beneficial for the support of larger cytoplasmic volumes, but there is not always a clear correlation between cell volume and ploidy (reviewed [33,34,74]).

Expression of CYCDs is excluded from differentiated and endoreduplicating tissues and is rather associated with proliferative tissues, such as meristems and organ primordia [35]. Indeed overexpression of CYCD3;1 actively inhibits endoreduplication [35], suggesting that normal G1 controls may not operate during the endoreduplication phase, as does overexpression of S-phase cyclin CYCA3;2 [22]. Moreover, since mitosis does not take place during endoreduplication, mitotic cycBs and CDKBs are not required. Indeed, the expression of these genes is downregulated, as is M-phase specific kinase activity. The FZR protein CCS52A is expressed in endoreduplicating cells, hence targeting mitotic cyclins for degradation, and downregulation of CCS52 results in reduced endoreduplication ([36]; reviewed by Kondorosi and Kondorosi [37]). Additionally, Wee1 expression is associated with endoreduplicating endosperm, inhibiting mitotic kinases via reversible phosphorylation [29]. Endoreduplication seems to therefore require inhibition or at least downregulation of normal G2 controls. Consistent with this overexpression of the mitotic cyclin CYCB1;2 in trichomes, unicellular three-pointed leaf hairs that normally endoreduplicate, resulted in multicellular trichomes [38]. Overexpression of CYCD3;1 also caused multicellular trichomes, the DNA content of each trichome is increased suggesting additional DNA replication as well as cell division, again consistent with the dual action of D-type cyclins as dominant drivers of the G1/S transition and as inhibitors of endoreduplication (Fig. 1). Indeed, expression of CYCD3;1 in trichomes induced endogenous CYCB expression, suggesting a mechanism for the inhibition of endoreduplication. Combined overexpression of both CYCD3;1 and CYCB1;2 enhanced the trichome phenotype, suggesting CYCB1;2, is nevertheless rate-limiting for mitosis [39].

Overexpression of CDK inhibitors blocks endoreduplication in both tissues and trichomes [27,40]. Similarly, Rb protein accumulates as leaves differentiate and as endoreduplication is initiated in endosperm. However, due to Rb-associated kinase in the endosperm, Rb is hyperphosphorylated and inactive. Elevated S-phase associated kinase activity was found in endoreduplicating tissues, consistent with continued DNA synthesis, although the kinase components were not identified (reviewed by Kondorosi and Kondorosi [37]; [41]). Therefore, overexpression of CKIs may block endoreduplication through their repression of the kinase activity required for DNA synthesis. In contrast, overexpression of CYCD3;1, although presumably increasing the kinase activity required for DNA synthesis, blocks endoreduplication through its simultaneous induction of mitotic kinase activity.

A limited number of cell cycle regulators enhance endoreduplication (Fig. 1). Elevated E2Fa/DPa activity promoted two additional rounds of endoreduplication in seedlings. Cells with large nuclei were observed in cotyledon palisade parenchyma, root cortex and trichomes, leaf epidermis, trichomes and spongy mesophyll [42,43]. Since E2Fa/DPa drives additional endoreduplication in some cell types, but mitosis in others, it has been suggested that the presence or absence of a hypothetical mitosis-inducing factor (MIF) plays a role [42]. It has been proposed that the role of MIF may be performed by the plant specific CDKBs. Interestingly, overexpression of the E2F target genes CDC6 and CDT1 increased ploidy levels, and CDC6 protein stability is higher in endoreduplicating tissues suggesting the involvement of SCF (Skp1, Cullin, F-box protein complex) in CDC6 turnover [44,45].

4. The shoot apical meristem: combined growth and organogenesis

The shoot apical meristem (SAM; Fig. 2) is formed during embryogenesis and contains the population of stem cells that give rise to all above-ground portions of the plant [46]. It also represents a major site of cell division. In dicotyledonous plants it consists of a shallow dome of cells at the centre of which is a group of slower-dividing cells that form the "central zone" (CZ) and represent the stem cell population [47]. Surrounding the CZ is the peripheral or organogenic zone (PZ), in which cells divide faster and organ primordia (leaves or flowers depending on the developmental stage) are initiated in a spiral phylotaxy (Fig. 2C) controlled by auxin signals [48].

The pattern of cell division within the SAM can be visualised by fusions of a marker protein to a cell-cycle regulated protein such as a CYCB [49,50], or by in situ hybridisation to cell cycle regulated probes [51]. Such analysis shows that divisions within the meristem are not synchronous, except possibly for daughter cells arising from an immediately preceding division, providing no evidence for an overall coordination of cell division. This argues for cell-autonomous decisions on commitment to mitotic division, and underlines the importance of the intrinsic regulatory mechanisms operating during G1-phase.

The CZ, PZ and organ primordia themselves are characterised by different patterns of expression of genes that control meristem behaviour. The stem cell population of the CZ is maintained by receiving a signal from cells immediately underlying them dependent on the product of the *WUSCHEL* (*WUS*) gene. The stem cells respond by producing a peptide signal encoded by *CLV3*, and this is perceived by the *CLV1*-encoded receptor in the *WUS*-expressing cells and limits the size of their population [75,76]. Hence, a mutually dependent feedback loop maintains both the stem cell population and the underlying *WUS*-expressing population. The expression of the *WUS*,*CLV1* and *CLV3* genes is tightly spatially regulated to the cells in these domains (Fig. 2D).

A further key meristem regulator is a homeobox gene known as *SHOOT MERISTEMLESS*, and *stm* mutants lack a functional shoot meristem [77]. *STM* expression is localised over the whole SAM, but is excluded from incipient and developing primordia [78]. Overexpression of *STM* strongly inhibits leaf cell expansion and differentiation, but does not affect primordia initiation itself, showing that the role of *STM*

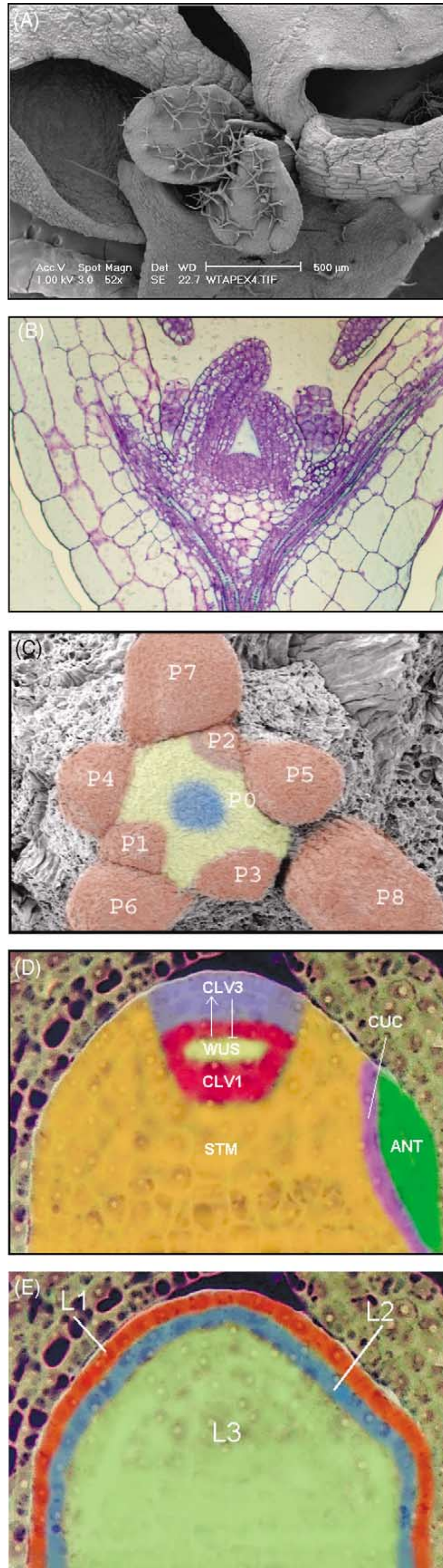

Fig. 2. The shoot apical meristem. The shoot apical meristem (SAM) is an important site of cell division, which drives post-embryonic development. (A) Scanning electron micrograph of the shoot apex of Arabidopsis. The two newly emerged leaves highlight the importance of the SAM in organogenesis. (B) A longitudinal section through the SAM, stained to show cell walls. Two emerging leaf primordia (LP) are indicated and reiterate the role of the SAM in leaf development. Larger more differentiated cells are seen in the cotyledon (seed leaf) bases surrounding the meristem. (C) Scanning electron micrograph of the reproductive SAM (above-view) depicting the zonation model of organisation. The central zone (CZ; blue) acts as a self-renewing stem cell population, while cells in the peripheral zone (PZ; yellow) are used for the initiation of lateral organ primordia. Organ primordia emerge successively in spiral phylotaxy (red). (D) Schematic diagram of expression patterns of *WUS*, *CLV1*, *CLV3*, *STM*, *CUC* and *ANT* in the SAM. Expression of *STM* (orange) also occurs within *WUS*/ *CLV* domains but is excluded from *ANT* expression domain (incipient organ primordia). (E) Longitudinal section of the SAM showing the L1 (red), L2 (blue) and L3 (green) cell layers.

appears to be that of maintaining the undifferentiated cellular state characteristic of meristematic cells [52,53]. Gradual loss of *STM* function by inducible *STM* RNAi leads to a failure to maintain the meristem and a loss of *WUS* expression (Scofield and Murray, unpublished data).

Despite the extensive knowledge of regulatory genes responsible for maintaining the meristem on the one hand, and of cellular regulation of division control on the other, little is known of the links between the organ and cell level in these processes. This remains an area of active and exciting research.

5. Leaf ontogeny

Over the meristem dome, the structure is maintained by the organisation of cells in layers, with cells in each layer normally dividing with their new cell walls perpendicular to the lateral neighbours (anticlinal divisions). In most SAMs, the outermost two cell layers (L1 and L2), consisting of the epidermis and layer below (L1 and L2), show this behaviour whilst below the L2 is a less organised L3 layer in which alternative division orientations also occur (Fig. 2E). The initiation of a leaf begins with the periclinal division of a cell in the L2 [46], which initiates the production of an outgrowth that will result in the leaf primordium through both increased proliferation of cells within the primordium and the recruitment of adjacent PZ cells [50]. In Arabidopsis, Traas and colleagues [54] have estimated that 30–50 cells are recruited into a leaf primordium. These changes of cell fate involved in producing a primordium are marked by patterns of expression of specific regulatory genes. In particular, *STM* and related homeobox genes are switched off in incipient and developing primordia. The primordium boundary with the bulk of the meristem is demarcated by a sheet of cells expressing the *CUP-SHAPED COTYLEDON* (*CUC*) genes [55], and which may also have ceased division. Interestingly, in *Antirrhinum*, different *CYCD3* genes are expressed in organ primordia and across the remainder of the meristem [56], indicating differ-

ences in proliferation control between cells in primordia and the meristem. Shortly after formation the primordium also starts to express differentially genes involved in determining the dorsal–ventral axes, such as *PHABULOSA*, *PHAVOLUTA*, *KANADI* and *YABBY* [79–81].

Once formed the outgrowth of the primordium and formation of the leaf blade itself is driven by two distinct processes—cell proliferation followed by cell expansion associated with differentiation and endoreduplication [57,58]. In the leaves of dicot plants these processes are separated temporally, with a phase of cell proliferation being primarily responsible for driving leaf growth until the size of a few millimetres, after which a progressive cessation in cell division proceeding from the distal tip towards the base is followed by the differentiation and expansion phase [58,26]. Most visible increase in size of the leaf from a few millimetres to mature size is therefore provided by cell expansion. In the leaves of monocots such as grasses, which are characterised by continued growth of the leaf blade, the separation of proliferation and differentiation is primarily spatial, with fully differentiated cells at the tip and a proliferative, mersitematic-like region at the base. A progressive decrease in CDK activity and increase in RB protein levels is seen progressively from base to the tip of growing maize leaves [59].

The altered expression of cell cycle regulators can have significant effects on the development of leaves, and indeed on other organs, with the effects observed depending on whether the perturbations are global or localised. Inhibition of division by expression in Arabidopsis of KRP2, a CDK inhibitor, leads to small, highly indented leaves. The indentations are due to a failure to complete the lamina by cell proliferation. The cells within the leaf become much larger than normal, suggesting a compensatory mechanism imposed by an overall regulation of leaf size, which attempts to make up for the short fall in cell number by increasing the degree of expansion of the available cells. However, endoreduplication is not affected either in its timing or extent [26]. Similar effects of ectopic KRP expression have also been observed in petal development [60].

Combined overexpression of the positive cell cycle regulators E2F and DP leads to ectopic activation of cycling, with promotion of both proliferation (mitotic) and endoreduplication cycles, suggesting that the particular E2F/DP combination used does not strongly affect the transition of cells from the mitotic to the endocycling phase but promotes cycling in both states [42].

In contrast, the D-type cyclin CYCD3;1 leads to multiple effects on leaf development by promoting mitotic cycles and also inhibiting endocycles. At the cell cycle level, it appears to push cells from G1 into S-phase, but the overall cell cycle length is not shortened since G2 is extended (Samland et al., in preparation; [61]). As a result there is an increase in the population of G2 cells and a decrease in the G1 population [35]. In the context of leaf development, this active promotion of cell cycling and inhibition of endocycles is accompanied by highly curled leaves consisting of relatively small, undifferentiated cells, which fail to exit the mitotic phase. As a consequence, the major phase of leaf growth is converted from an expansion driven to a proliferation-driven process (Fig. 3). Again, the maintenance of general leaf architecture

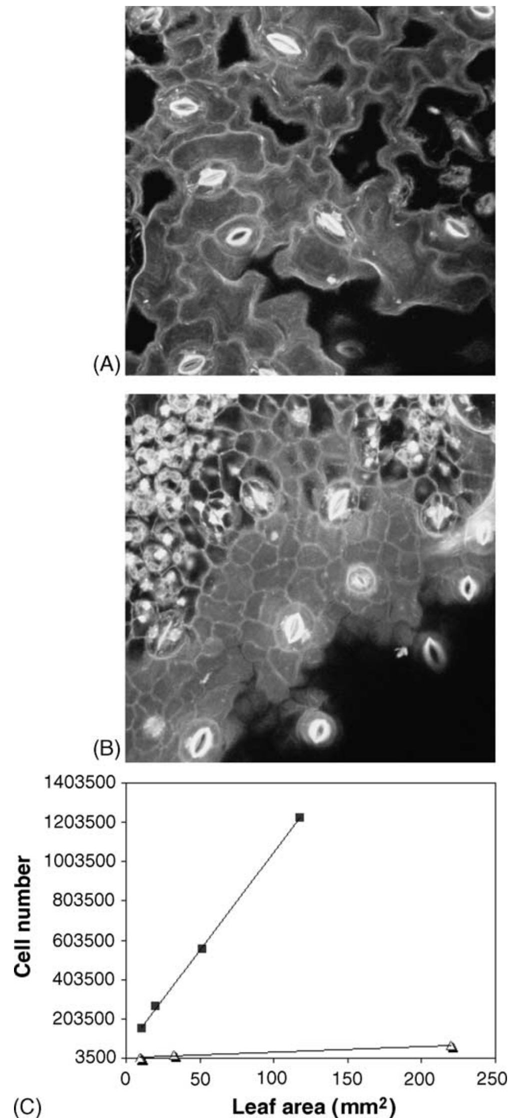

Fig. 3. Cyclin CYCD3;1 promotes cell division driven development. (A) Optical section of the surface (epidermis) of a wild-type Arabidopsis leaf, showing several stomata (bright ovals) and the complex sinusoidal outlines of the epidermal cell walls. (B) Equivalent view of epidermis of leaf of CYCD3;1-overexpressing Arabidopsis plant line. Note that the formation of stomata is not affected, but the surrounding epidermal cells are small and much more rounded in outline, characteristic of less differentiated cells. Endoreduplication of these cells is also strongly reduced (not shown). (C) Overexpression of CYCD3;1 switches leaf growth from a primarily cell expansion driven process to one driven mainly by cell division. Graph shows total leaf cell number against leaf area, showing that in wild-type leaves (lower line) increase in size is not accompanied by a concomitant increase in cell number. In plants with ectopic expression of CYCD3;1, leaf area increase is directly proportional to cell number. The final leaf size is approximately half that of wild-type leaves but containing 20-fold more cells. For detailed analysis see [5].

in the context of a paradigm shift in the mechanism of organ growth argues for overall controls of organ shape and size operating above the level of cell processes. Similar to the effects of CYCD3;1, overexpression of CYCA3;2 promoted the G1/S transition and reduced endoreduplication [22].

Interestingly, localised effects on cell proliferation in the developing lamina, achieved by gene induction using the placement of beads on primordia, showed that these controls operate at a global organ level rather than independently on different regions of the organ independently. Fleming and co-workers [62,63] showed that localised induction of cell division resulted in an *inhibition* of localised growth of that region of the leaf, and conversely decreased division led to an increase in the relative growth of the affected region. These apparently counter-intuitive results may reflect the requirement for cell proliferation and expansion phases to be co-ordinate across the organ, and that a domain in which cell division is forced to continue for longer partially misses out on its subsequent opportunity for cell expansion.

Overall the results obtained from analysis of cell division in the formation of leaves shows a progressive differentiation as cells pass through first a mitotic proliferative phase, followed by cell expansion and endocycling and an eventual cessation of all cell cycle activity and terminal differentiation. Organ level controls attempt to maintain size and architecture within the limitations of the cellular building blocks available. This provides the framework within which perturbations of cell division can be interpreted.

6. Root development: spatial separation of growth and organ initiation

The root apical meristem (RAM; Fig. 4A) is situated close to the tip of the primary root, and consists of a proximal proliferative zone and an elongation zone situated behind the meristem proper. The combined processes of division and expansion provide the growth of the root. The RAM shows a more obvious structural organisation than the SAM, with a small group of slowly dividing stem cells known as the quiescent centre giving rise to initials corresponding to each of the cell files in the mature root. Division occurs mainly of the initial cells and their immediate progeny, and the plane of division is almost always perpendicular to the root axis leading to the maintenance of cell files. However, careful laser ablation studies show that despite the lineage relationships within cell files, cell identity is determined by position, not lineage, and ablated cells can be replaced by divisions from neighbouring files [64].

The SAM and the RAM represent alternative modes of lateral organ initiation, in the first case involving the setting aside of specific cells within the primary meristem which will develop into a new organ, whereas in roots the formation of laterals involves non-cycling cells becoming re-initialised for cell division.

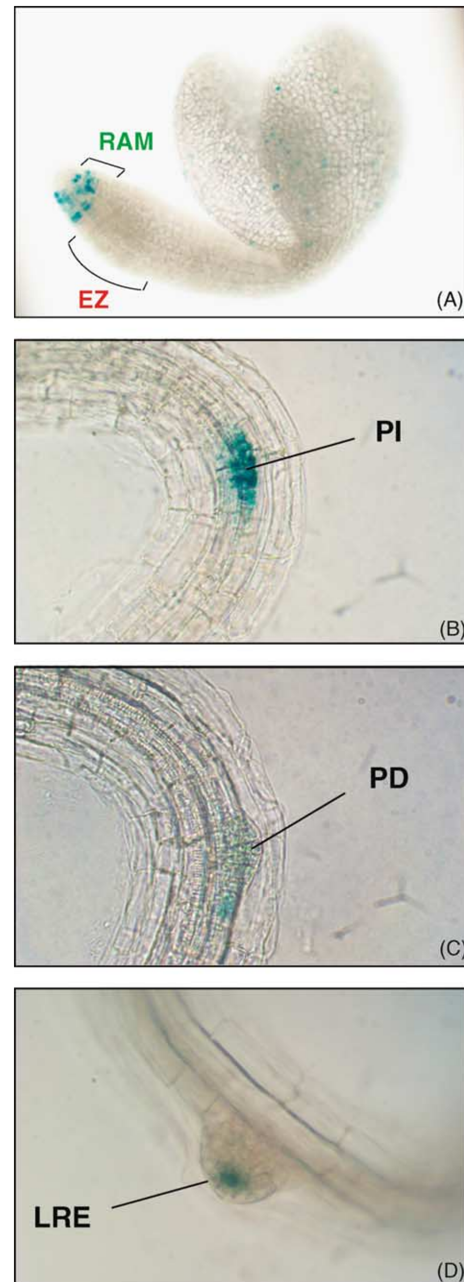

Fig. 4. Root development in Arabidopsis requires controlled cell divisions. (A) The root apical meristem (RAM) is demarcated by the staining of dividing cells in an imbibed embryo by using a fusion of the GUS enzyme to a mitotic (CYCB1) cyclin. In the root, cell position reflects cell age and hence older cells, which are not actively dividing, are found acropetal to the RAM in the elongation zone (EZ). Within the EZ, distinct pericycle cells give rise to lateral roots, which occurs through seven developmental stages [65]. This process can be more broadly described by three different periods of cell divisions which can be visualized by the GUS::CYCB1 reporter. (B) Lateral root primordium initiation (PI) is seen with early stage cell divisions creating the first 3 cell layers. It is believed that this process first requires the downregulaton of *KRP2* allowing the initial cells to divide (see text for details). (C) More cell layers are formed as cycling continues while other cells elongate and the primordium develops (PD) into the familiar bell-shaped structure and pushes through the endodermis. (D) Finally the new meristem is set up with actively dividing cells at the lateral root tip (recapitulating the RAM) facilitating lateral root emergence (LRE) through the epidermis.

Lateral roots develop from the initiation and maintenance of de novo meristems. These meristems arise from specific cell files within the root cell layer surrounding the vascular stele known as the pericycle. Upon an appropriate signal, a small number of pericycle cells undergo asymmetric transverse divisions [65]. These lateral root initial cells continue dividing periclinally, thus forming the characteristic bell-shaped lateral root primordium (reviewed by Malamy and Benfey [65]). This process involves discrete stages and is illustrated in Fig. 4B–D.

The Arabidopsis root has distinct cell cycle regulation. Initially, the entire embryonic root consists of cells held in G_1 (Matole and Murray, unpublished data). During germination, distinct waves of cell division occur and give rise to the different cell files including the pericycle. Recent data has shown that pericycle cells have distinct cell cycle identities based on position [66]. Using different Arabidopsis lines carrying G_1- and G_2-specific cyclin::*GUS* gene reporters, Beeckman et al. [66] showed that most pericycle cells are held in G_1 except the xylem pole pericycle cells which progress through S-phase and remain in G_2, primed for lateral root initiation. These cells are then re-activated for cell division, creating a secondary meristem and hence a lateral root primordium. What then is the signal for the primed cells of the pericycle to begin dividing and which cell cycle related factors are specifically needed to initiate lateral roots?

For some time auxin has been implicated in lateral root growth due to the altered lateral root numbers in auxin mutants. For example, in mutants that are defective in auxin sensing such as *slr*/*IAA14*, *axr1* and *aux1* lateral root numbers are reduced, whereas in the *superroot* mutant, which has increased endogenous auxin levels, lateral root numbers are increased [67–70]. More recently, auxin has been shown to be both necessary and sufficient to initiate lateral root formation [71]. Germination on an auxin transport inhibitor, NPA, prevents the formation of lateral roots, most likely by preventing the priming of pericycle cells in the cell files opposing the xylem poles. However, upon transfer to media containing auxin, the entire pericycle cell file opposite the xylem poles is activated and lateral roots are synchronously initiated. Using this system, Himanen et al. [71] characterised the transcriptional control of cell cycle genes during the early development of lateral roots and showed that *KRP1* and *KRP2* expression decreased shortly after auxin induction of pericycle cells presumably relieving negative regulation of G_1. The importance of this was confirmed by decreased lateral root numbers in Arabidopsis plants ectopically expressing *KRP2*. Subsequent to *KRP1/2* downregulation, various G_1- and G_2-specific cyclins, including *CYCB1;1* and *CYCD3;1*, are upregulated, presumably facilitating continued cell divisions in the lateral root primordia and hence allowing growth of the lateral root. A more robust transcript profiling experiment using the lateral root inducible system has recently been performed corroborating these experiments [71]. The authors reported that *KRP2* transcripts decreased shortly after auxin treatment while G_1/S specific transcripts (E2Fa and histone H4) became more abundant. Later stage expression consisted mostly of G_2/M cell cycle genes including *CDKB1;1*, *CYCB1;1* and *CYCB2;1*. From these experiments they conclude that lateral root development in this system has three phases. Initially, cells are blocked in G_1 probably by the KRP proteins, especially KRP2. Then upon an auxin signal, this block is removed and cell cycle components are expressed driving cells through the G_1/S boundary. Finally, G_2/M specific components are expressed and the pericycle cells begin dividing, facilitating lateral root primordia growth. It must be cautioned, however, that this system activates the entire xylem pole pericycle cell file resulting in ectopic lateral roots and hence subtle differences, especially in initial cell cycle regulatory events, between lateral root initial cells and the rest of the pericycle may be obscured. Indeed previous experiments had indicated that primed lateral root initials remain in G_2 and are not held in G_1 as suggested by the transcript profiling [66]. In light of this, germinating seeds on NPA may be blocking all cells of the pericycle in G_1 before proper priming occurs thereby removing the developmental context of the system.

Cyclins are important mediators of cell cycle progression (see above) and they are known to be expressed in the pericycle and developing lateral root primordia [66]. Thus, cyclins represent good candidates as factors contributing to lateral root initiation. Early studies had identified *CYCB1;1* (a G_2-specific cyclin) as a cyclin that is expressed in pericycle cells during lateral root initiation. However, using lines to ectopically express *CYCB1;1* resulted in no difference in lateral root numbers indicating that this cyclin was not limiting for lateral root formation [47]. Similarly, plants overexpressing *CYCD3;1* do not appear to have altered lateral root numbers (Dewitte and Murray, unpublished data). Another candidate is the *CYCD4;1* which is expressed at the boundaries of the lateral root primordia and is able to bind both G_1- and G_2-specific CDKs [72,3]. However, while cell cycle regulation is thought to be substantially achieved by transcriptional controls, no alterations in the transcription of *CYCD4;1* was observed in the transcriptional analysis of lateral root initiation [71]. Thus, lateral root development may depend upon temporally regulated kinase activity that is not dependent upon any one specific cyclin component per se. Supporting this is the potential redundancy between plant cyclins, which is consistent with the overlap in expression between many of them [7]. In addition, post-transcriptional controls on cyclin/CDK complex activity during lateral root initiation may be significant. Consistent with such mechanisms is the identification of *KRP2* as an upregulated component in pericycle cells. Also cyclin stability, perhaps by auxin regulated ubiquitin-directed protein degradation (a process known to be associated with lateral root development, reviewed by [73]) may contribute to the regulation of kinase activity in these cells. Indeed evidence on CYCD3;1 turnover supports this as an important mechanism controlling cyclin activity [8].

7. Concluding remarks

Plants represent fascinating systems for study of the relationships between cell cycle control, differentiation and development, because plants exhibit indeterminate growth and hence have close integration of division processes into the whole of a life cycle, which is itself plastic and responsive to outside environmental controls. Indeed the post-embryonic increase in size and organ number can be very substantial in the case of large and long-lived plant species. Moreover, within a single organism, different models of organ initiation can be seen, exemplified by leaf and lateral root initiation, but a common theme is the spatial and/or temporal separation of cell division and cellular differentiation within developing organs. The structure and function of plant meristems is intimately linked with the division processes within them in ways that are yet to be unravelled. This understanding is of great significance, since at a profound level all terrestrial life depends ultimately on the activity of plant meristems.

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