

What's New in the Plant Cell Cycle?

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Abstract The cell cycle comprises mitosis, post-mitotic interphase (G1), DNA synthetic phase (S-phase), post-synthetic phase (G2) and mitosis (M). In this review, new papers on the plant cell cycle (2007–2008), where the authors have focused on the critical transitions of the plant cell cycle, G1/S and G2/M, have been highlighted. Interestingly, disruption of one subset of cyclin-dependent protein kinases (a CDK B-type) disrupts the normal functioning of shoot but not root apical meristems in Arabidopsis. XAL1 is an agamous-like protein that has a differential role in roots compare with shoots; *xal1* mutant alleles have reduced root meristem size and lengthened cell cycles. E2F proteins are transcription factors that drive cells into S-phase. E2Fc may be targeted for degradation by the molecular machinery leading to the 26S proteasome. The Retinoblastoma (Rb) protein is a negative regulator of E2F. New evidence is also presented whereby starving plant cells of sucrose did not affect expression of Rb which would be consistent with an arrest of those cells in G1 in these conditions. Dynamin-related proteins are

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important at cytokinesis, and recent evidence suggests that one subset plays a crucial role in cell wall synthesis. These new papers are reviewed against a background of what is currently known about regulation of the plant cell cycle.

1 Introduction

The cell cycle is a series of events that a proliferative cell must traverse before it can divide. Classically, the cell cycle is separated into four successive phases: mitosis (M), G1 (post-mitotic interphase), S phase (DNA Synthetic phase) and G2, post-synthetic phase (Fig. 1).

The major transitions are G2 to M, when proliferative cells achieve mitotic competence, and G1 to S-phase, when cells gear-up for nuclear DNA replication. However, in reality, the cell cycle ensures correct timing of both DNA replication and mitosis. If timing begins to malfunction in cells that are deficient in core cell cycle genes, catastrophic down-spiralling of cell size or unscheduled mitoses could follow. There is some published literature on the plant cell cycle that assigns a cell cycle transition as a checkpoint. In my view, checkpoints are stress-induced blocks on the cell cycle that a cell must clear before undergoing the transition. Also, and as clearly noted by O'Connell et al. (1997), a checkpoint gene is only expressed in response to a stress (e.g. UVB). Mostly, in this review I consider what is new in the "normal" cell cycle, if such a condition actually exists.

Perhaps not surprisingly, given the universality of DNA, the basic events of the cell cycle are common to all eukaryotes. All proliferative cells must replicate their nuclear DNA before they are competent for mitosis. It therefore follows that many of the genes that regulate the G2/M and G1/S transitions are similar among all eukaryotes. However, there are certain aspects of the plant cell cycle that are unique. For example, cytokinesis is very different between plants and animals. During plant cytokinesis, a new cell wall is formed between two recently divided

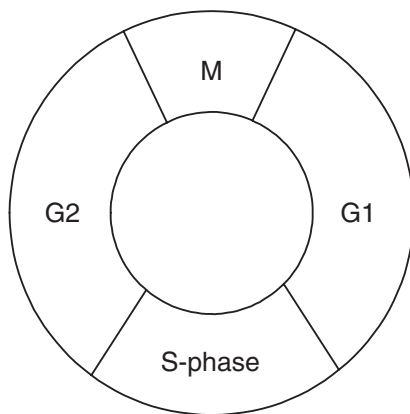


Fig. 1 An idealised cell cycle comprising G1, S-phase, G2 and M

cells. For this to occur, unique plant genes are expressed and structures necessary for cytokinesis are formed that do not have a counter player in animal cell cycles. Another difference is that either G1 or G2 are natural arrest points in the plant cell cycle whereas in animals, G1 is the natural arrest point.

In this short review, the aim will be to paint a general picture of the plant cell cycle and to highlight, in the author's opinion, what is relatively new. The reader can also be updated via two very recent publications on the plant cell cycle (Inze 2007; Bryant and Francis 2008).

2 Cyclin-Dependent Kinases and Cyclins

The major phase transitions of the cell cycle, G1/S and G2/M, are driven by cyclin dependent protein kinases (CDKs). Currently 152 CDKS have been identified from 41 plant species (Dudits et al. 2007). In animals, CDKs are numbered whilst plant CDKs are suffixed by Arabic letters in seven classes (A to G) along with an eighth rather more anomalous class, the CDK-like kinases (CKLs). CDKs phosphorylate substrates to drive a cell through a particular phase transition. CDKA was first cloned in *Arabidopsis*, *Arath*;CDKA;1, but originally was known as *Atcdc2a* (Ferreira et al. 1991). The gene could complement a *cdc2*⁻ mutant of fission yeast, and its encoded protein exhibited a highly conserved sequence of amino acids, the so-called PSTAIRE motif in its α 1 helix. A second *Arabidopsis* CDK (*cdc2b*), now known as one of the *Arath*;CDKB types was identified, but it cannot complement the yeast *cdc2* mutants and PSTAIRE is replaced by PPTALRE reflecting evolutionary divergence between the B and A-types (Segers et al. 1996). It was subsequently discovered that the B-type was a member of quite a large family of CDKs that are unique to plants (Dudits et al. 2007). However, despite the ever-growing number of plant CDKs rather little is known about their substrate specificity, and the picture is not much clearer in animal cell cycles in this regard.

CDKs, C and G, may or may not operate in the cell cycle and much more needs to be learnt about them. Arguably, therefore most is known about the A- and B-type CDKs. *Arath*;CDKA;1 is expressed more or less constitutively both with respect to the cell cycle and different regions of the plant, and its encoded kinase activity is highest at G1/S and G2/M in *Arabidopsis*, whilst in the tobacco BY-2 cells, CKDA activity is relatively constant from S-phase to G2/M (Joubes et al. 2000). In contrast, B-type CDKs are transcriptionally regulated during the cell cycle and their expression tends to be limited to the meristems of the plant (Ferreira et al. 1994). In BY-2 cells, CDK kinase activity peaks in G2 (Sorrell et al. 2001).

For some time now, the consensus view has been that a functional link between cell cycle genes and development of the plant is tenuous. For example, a dominant negative *Arath*;CDKA;1 allele when expressed in tobacco resulted in fewer but larger cells, but the development of those plants was not unduly affected (Hemerly et al. 1995). Plants expressing a DN allele of *Arath*;CDKB1;1 also displayed a normal shape, but again with fewer and larger cells with long G1 phases (Porceddu et al. 2001). Thus, the data were consistent with a null effect of cell cycle manipulation on development. Indeed, the data support the organismal theory of development where cell division is merely a

functional consequence of inherent developmental programmes (Kaplan and Hagemann 1991). However, recently, a disruption of *CDKB2* led to impaired cell cycle progression and induced severe defects in the shoot apical meristem of *Arabidopsis*. Also, evidence was provided that *CDKB2* is a necessary part of a coordinated programme of gene expression including *WUSCHEL* and *SHOOTMERISTEMLESS* that is necessary for correct functioning of the vegetative shoot apical meristem (Andersen et al. 2008). Indeed, these data are consistent with a requirement for meristem organisation and cell division to be carefully coordinated for normal shoot apical meristem function. As I have stated several times before, the organismal and cellular theories of development are unwisely portrayed as mutually exclusive hypotheses. I agree with the recent conclusion of Wang et al. (2008), from their studies of inhibitors of CDK activity (see Sect. 6), that cell division, growth and development are intrinsically intertwined.

3 Cyclins

CDKs only have catalytic activity when bound to a partner cyclin and cease to be catalytic following an almost instantaneous destruction of the cyclin. This was first discovered in sea clams as a protein that increased in concentration as meiocytes prepared for meiosis and then suddenly disappeared when these meiotic cells began to divide (Evans et al. 1983). This well-timed destruction is best characterised for cyclins that degrade in anaphase of mitosis. Plant cells cannot complete mitosis normally if CDK activity persists beyond anaphase (Weingartner et al. 2004). The timing of G1/S cyclin degradation is less instantaneous than that of the G2/M cyclin at anaphase (Morgan 2007) although, to my knowledge, there are few data on the kinetics of cyclin degradation in plants.

In lower eukaryotes, such as budding and fission yeast, there is one CDK (Cdc2 in fission yeast and CDC28 in budding yeast) that binds with different cyclins at different stages of the cell cycle (Morgan 2007). However, in animals and plants, different cyclins and different CDKs function at different points in the cell cycle (Table 1).

Table 1 A generic listing of cyclin-dependent kinases (CDKs) in higher plants (for a comprehensive listing see Dudits et al. 2007) together with likely plant cyclin partners (for an up-to-date listing see Nieuwland et al. 2007)

	G1/S	"Likely cyclin partner	G2/M	
CDKA	✓	D/A (P?)	✓	D/A
CDKB		B (P)	✓	B
CDKC		C/T ¹		Phosphorylation of C-terminal of large sub-unit of RNA polymeraseII
CDKD		H		CAK kinase
CDKE		?		?
CDKF		?		CAKAK kinase
CDKG		?		?

¹John Doonan, personal communication

In plants, over 100 cyclins have been identified and are grouped according to their homology with animal cyclins which themselves have been apportioned into 13 classes (A–L and T). In *Arabidopsis thaliana*, the A-, B- and D-type cyclins are important during the cell cycle but there are a host of others identified, including C-, H-, L-, P- and T-types which may or may not be integral to the cell cycle (Table 1).

4 G0/G1/S-Phase

Like animal cells, plant cells will arrest in G1 with the 2C amount of nuclear DNA. They do this if deprived of nutrient (Van't Hof 1966) or if stressed in other ways such as low temperature (Francis and Barlow 1988). Such non-cycling cells are said to be in G0. A stunning example of G0 cells in the plant is the quiescent centre in root apical meristems (RAMs). First predicted and then discovered by Clowes, this is a tiny population of cells; for example, 300 out of 250,000 in broad bean RAMs (Clowes 1967) and as few as four in *Arabidopsis* (van den Berg et al. 1997). These cells show low rates of DNA, RNA and protein synthesis (Clowes 1956). In higher plants, it was hypothesised that G0 cells are located in a central position within the QC, whereas on the margins of the QC there are slowly cycling cells with very long G1 phases. Indeed, these very slowly proliferating cells have been identified in RAMs of *Zea mays* (Clowes 1970). When the root cap is damaged, cells of the QC spring to life and rapidly repopulate the cap before returning to their quiescent or slow cycling status (Barlow 1974). Such a population of cells conforms to what animal cell biologists would call stem cells. To have stem cells in RAMs has always seemed to be botanically bizarre (at least to this author!) and maybe we should rename them as founder cells.

Some time ago, Feldman elegantly showed that the QC could be excised and cultured, and from which an entire plant could be regenerated (Feldman and Torey 1975). These observations were consistent with the idea that cells of the quiescent centre are pluripotent. However, and at the time somewhat surprisingly, when all cells of the QC were laser ablated, subtending cells of the cortex were recruited into a rapidly reconstituted QC. Hence the QC and contacting apical initials proved to be surprisingly plastic. Moreover, if single QC cells were laser ablated, cells in direct contact with the now defunct QC cell accumulated starch, leading to the conclusion that cells of the quiescent centre inhibit the differentiation of surface-contacting initial cells (van den Berg et al. 1997). How exactly quiescence is imposed on QC cells is unknown, although the recent discovery of a homeodomain *WUSCHEL*-like gene, *QHB*, would suggest feed-back loops that involve plant growth regular-induced signal transduction chains that stabilise the population of QC cells (Kamiya et al. 2003).

Recently, a MADS-box gene, *XAL1*, was shown to be involved in regulating root growth. *xall* mutant alleles had reduced meristem length, increased cell cycle duration and reduced rates of cell production compared with wild type. The results were surprising given that *XAL1* is very closely related to the

AGAMOUS MADS-box clade that specifies carpel morphogenesis. The authors proposed that *XAL1* may have a dual role in RAMs and SAMs through a differential coupling with different types of hormonal signals that specify root or shoot morphogenesis (Tapia-López et al. 2008). In my view, this is a very nice example of a homeodomain gene interfacing between developmental, cell cycle and growth controls and a further reminder of distinct differences in the functioning RAMs and SAMs.

4.1 *G1/S Cyclins*

As in animal cells, D-type cyclins are to the fore in activating cells from G0 to G1 (Menges and Muray 2008). Interestingly, the latter authors took one step back and considered the issue of arrest in G0 induced by stress factors. It transpires that expression of at least one member of the cyclin classes: A-, B-, C-, D- P and H are down-regulated in response to heat stress. We now begin to get a clue about why that there are so many cyclins in plants compared with animals. It seems that this array of regulatory proteins form part of the plasticity of responses that plants must have in order to cope with unpredictable changes to the environment. Indeed, the sudden stops and starts to plant growth in the wild must be at least partly governed by very fine tuning of plant cyclin gene expression.

4.2 *E2F*

The E2F-DP family of transcription factors plays a big part in activating cells into S-phase. As is often the case, the abbreviations of genes can have a complicated history that bares only a passing relationship to the function the protein in question. E2F is no exception. It was first identified when a promoter of a human adenovirus, E2, was activated by a host cytoplasmic binding factor (protein) (Dyson 1998). DP is a dimerisation protein, a comparatively simpler abbreviation. Currently, three plant E2Fs are well characterised (a, b and c) and there are at least three more varying in nomenclature from E2F d, e through to DEL1 2 and 3, and they may not require a DP to function normally (Ramirez-Parra et al. 2003, 2007).

Many genes have E2F recognition sites in their promoters, and the general picture is that E2F is necessary to activate S-phase genes that drive cells into nuclear DNA replication (Ramirez-Parra et al. 2003). Recently, in *Arabidopsis*, E2Fc, which is a putative negative regulator of other proteins, was shown to be targeted for degradation by SKP2A (Jurado 2008). The latter protein is part of a rather larger complex, SKP/CULLIN/F box complex that targets and steers proteins to the 26S proteasome for degradation (del Pozo et al. 2002a, b, 2006). *SKP2A* over-expression can stimulate cell division in meristems, but this might be because it targets E2FC for degradation (Jurado et al. 2008).

4.3 *Retinoblastoma Protein*

The E2F transcription factors are regulated negatively by the so-called retinoblastoma protein; there is one RB in *Arabidopsis* and several related (RBR) ones. At the G1/S transition, RB is hyperphosphorylated by CDKA that binds to several D-type cyclins (Bonnioti and Gutierrez 2001); this high level of phosphorylation destabilises binding between RB and E2F and the latter is then released to activate S-phase genes. Whether RB can regulate the number of founder cells in the quiescent centre of RAMs (see above) was tested recently. A local reduction in RBR expression resulted in an increase in the number of founder cells whilst RBR over-expression had the converse effect before ultimately arresting mitotic cells. They measured the frequency of mitotic figures in *Arabidopsis* roots and concluded that manipulation of RBR did not affect cell cycle times (Wildwater et al., 2005). However, it must be noted that the mitotic index is only a sensitive measure of rates of division if M-phase remains constant, and that any changes in the cell cycle time are due to changes in other component phases of the cell cycle.

A very well-known phenomenon in the plant cell cycle is that depleting cells of sucrose results in their natural arrest in either G1 or G2 (Van't Hof 1966). One feature of sucrose starvation in *Arabidopsis* cell cultures is that high expression of *Arath;RBR1* persisted despite the depletion of sucrose from the medium (Hirano and Sekine 2008). What this implies is that *Arath;RBR1* cannot be hyperphosphorylated and, as such, would remain bound to E2F. This would fit with down-regulation of D-type cyclins and as a consequence a down-regulation in CDK activity in cells that have been starved of sucrose (Murray et al. 1998).

5 S-Phase

During S-phase, DNA is replicated semi-conservatively so that the DNA molecule of a one-armed chromosome is unwound and each unwound strand acts as a template for replication of nascent DNA. S-phase terminates when two DNA molecules are fully replicated and housed in double-armed chromosomes. If only it was as simple as that!

DNA replication along the chromosome is achieved by firing of initiation points spaced at regular intervals along the chromosome. We calculated that 30,000 initiation points function to replicate all 14 chromosomes in a diploid rye genome, and this increased to over a million in the 42 chromosomes of the allohexaploid genome of *Triticum aestivum* (AD Kidd, D Francis, MD Bennett, unpublished data). An initiation site and its two termini span a length of DNA known as a replicon (Vant'Hof 1985). However, there is no evidence to support a temporal and controlled activation of successive replicons along the chromosome. The situation is much more complicated with different clusters of replicons firing at different times during S-phase. So, we have a perplexing problem that, although DNA replication is continuous from one initiation site (at least in the leading strand!), DNA replication is

most likely to be discontinuous on any given chromosome at any specific point during S-phase.

Eukaryote DNA contains genic DNA interspersed at random between vast domains of non-genic DNA. However, although the amount of DNA to be replicated is typically vast, S-phase can last for a staggeringly short period of 10min in *Drosophila* embryos, to several hours in species with very large genome sizes (Francis et al. 2008). The plasticity of S-phase can be explained partly by the activation of many replication origins for fast S-phases and by the quiescence of weak replication origins between strong ones as S-phase gets longer. Unlike budding yeast, there appear to be no specific sequences of DNA that configure a replication origin in higher eukaryotes, although an origin recognition complex (ORC) of six polypeptides typically binds to and is characteristic of a DNA replication origin. In budding yeast, a detailed but not necessarily complete map of proteins that interact to replicate nuclear DNA has been characterized, but space does not permit a detailed description of them (Diffley and Cocker 1992; Diffley and Labib 2002; Diffley 2004). Recently, some of the components of this network have been cloned in plants but currently we lack functional evidence for their role *in planta* (see Table 2).

Table 2 An array of well-characterised yeast and animal genes/proteins known to participate in the initiation of DNA replication together with known plant orthologues whose role *in planta* remains to be confirmed

Budding yeast gene	Type of protein	Function or probable function	Plant orthologue
ORCs ^a	Each ORC comprises six non-catalytic (structural (?)) polypeptides	Guard the replication origin	✓
CDC6 ^b	AAA ATPase	Attaches to ORC and “facilitates” binding of MCM proteins to ORC	✓ but max. expressed in early S-phase in BY-2 cells
CDT1 ^c	Binding protein	Attaches to ORC to assist initiation DNA replication but must be degraded to prevent re-replication genome	✓
MCM10	An essential S-phase protein	Required for initiation of DNA replication but <i>not</i> part of pre-replication complex (PRC)	
MCM2–7 ^{d–f}	Bind either side of ORC	“Have helicase-like activity facilitating” unwinding of DNA at the replication origin (part of PRC) although not when purified	✓
CDC7	Protein kinase	Phosphorylate ORC and MCMs enabling proper function of MCMs and binding of additional proteins of the replication complex (RC)	

(continued)

Table 2 (Continued)

Budding yeast gene	Type of protein	Function or probable function	Plant orthologue
DBF4	Regulatory subunit	Functions alongside <i>CDC7/DBF4</i> ✓ to trigger initiation of DNA replication after the PRC has formed	
CDC45 ^g	Binding protein		
GINS1–4	Binding proteins factor	Most likely stabilise and promote helicase activity of MCMs in the RC	
SLD	Binding proteins	Part of RC aiding DNA unwinding and replication	

^aGavin et al. (1995)^bRamos et al. (2001)^cLin et al. (1999)^dBryant et al. (2001)^eSpringer et al. (1995)^fSabelli et al. (1996)^gStevens et al. (2004)

6 G2/M

During mitosis, an exact partitioning of chromosomes occurs so that two identical sets of chromosomes become housed in new nuclei in smaller cells. Once complete, the growth cycle begins once more as the new cells prepare for the next mitosis. Hence, G2/M is a critical transition in the life of the cell. Unerringly, most cells of a healthy organism undergo mitosis correctly at each and every mitosis. This is despite environmental insults to DNA of the staggering order of 2×10^5 – 4×10^5 single strand breaks cell⁻¹ day⁻¹ (Bray et al. 2008). The clear picture in animals and the emerging one in plants is that cells have multiple mechanisms to stop themselves from dividing inappropriately, but have only a fleeting opportunity to divide providing every aspect of mitotic competence is fulfilled. The inhibitory pathways will be explored here.

6.1 Negative Regulation by Lack of Cyclins

At the biochemical level, a mitotic CDK can be switched off quite simply through lack of the partner cyclin. Mitotic cyclins peak in concentration at G2/M and are degraded in anaphase of mitosis. The dependency on the correct timing of cyclin synthesis and degradation was aptly demonstrated when *Arabidopsis* was transformed with a mitotic cyclin lacking a destruction box. This created a transgenic

line with non-degradable cyclin. The G2/M, prophase and metaphase stages were completed normally, but both anaphase and telophase became chaotic as the mitotic cells lurched into aberrant divisions (Weingartner et al. 2004). Hence, not only is the carefully timed activation of CDK activity critical for G2/M but its inactivation by a well-timed destruction of the partner cyclin is also equally important for a successful mitosis.

The activation of binding between a cyclin and its CDK is regulated by phosphorylation of the CDK at a threonine residue (160/167) towards the carboxy terminus of the protein. This phosphorylation is now known to be catalysed by a cyclin-dependent activating kinase (CAK). In plants, this function seems to be performed by D-type CDKs which themselves are activated by an H-type cyclin (Table 1). In turn, the binding between CDKD and cyclinH is catalysed by CDKF which in effect is CAK activating kinase (reviewed by Dudits et al. 2007). One could imagine that this phosphorylation cascade operates indefinitely as there might be a previous activation between a CAK and its partner cyclin that is phosphorylated by another CAK, and so on. If so, the mother of all CAK kinases is unknown (in both plants and animals). However, it is probably the case that the CDK-cyclin complex, once activated, is capable of phosphorylating and activating an up-stream CAK kinase in a positive feed-back loop, although evidence of this in plants still awaits us.

6.2 *Negative Regulation by WEE1*

In most eukaryotes, another form of repression of CDKs at G2/M is exercised by WEE1 kinase. This kinase phosphorylates the CDK at its tyrosine15 residue close to the amino terminal of the CDK. In fission yeast, there is functional redundancy between WEE1 and MIK1 kinase (Lundgren et al. 1991). In humans, WEE1 kinase activity is high during interphase but drops in mitosis (McGowan and Russell 1995). Furthermore, CDK-cyclinB1 is partitioned away from the nucleus in interphase where its kinase activity is repressed by another Wee1-like kinase, MYT1 (Mueller et al. 1995), which phosphorylates both Thr14 and Tyr15 of CDK1 (the latter is an orthologous to *Arath;CDKA;1*). Hence, there is a double check on repressing CDK, keeping it out of the nucleus in interphase whilst WEE1 remains nuclear-located, capable of preventing or suppressing any promiscuous CDK activity during interphase (Baldin and Docommin 1995). At G2/M, CDK1-B1 enters the nucleus, WEE1 is partitioned into the outer reaches of the cytoplasm and, seemingly, any residual nuclear WEE1 is hyperphosphorylated by the CDK prior to proteolytic destruction of WEE1 (Watanabe et al. 2005). During interphase, nuclear WEE1 is tethered to a 14-3-3 protein which shields the WEE1 kinase from hyperphosphorylation and might also be important for the partitioning and movement of WEE1 from nucleus to cytoplasm at prophase of mitosis (Morgan 2007). And so, for the proliferative cell, we have an almost perfect inverse spatial and molecular relationship between WEE1 and the CDK, which prevents unscheduled CDK activity from initiating mitosis in unfavourable conditions.

The current status of *WEE1* in plants is far from clear. *WEE1* orthologues have been cloned in maize, *Arabidopsis* and rice (Sun et al. 1999; Sorrell et al. 2002; Guo et al. 2007). In *Arabidopsis*, *WEE1* expression is limited to proliferative regions of the plant whilst its over-expression in fission yeast induces a long cell phenotype, a classic screen for *WEE1* orthologues (Sorrell et al. 2002). Moreover, the plant *WEE1* kinase is active in interphase but is very low during mitosis, and it can bind to Non-Epsilon plant 14-3-3 proteins in a cell cycle dependent manner (D. Francis et al., unpublished data). However, *Arath*;*WEE1* could not complement a *wee1/mik1* double mutant of fission yeast (Sorrell et al. 2002). Also, a *WEE1* T DNA insertion line (*wee1-1*) develops normally and *WEE1* itself was only highly expressed in *Arabidopsis* plants treated with hydroxyurea (De Schutter et al. 2007). The latter perturbs DNA replication by competing with endogenous nucleotide pools within the cell. The conclusion drawn from this work is that *WEE1*'s role in the plant is restricted to stress responses so that an over-expressed *WEE1* will prevent cells from dividing until DNA damage is repaired or DNA replication is normalised. We have unpublished data (manuscript submitted for publication) indicating that *wee1-1* does exhibit a subtle root phenotype, so that perhaps *WEE1* is an integral component of the processes that governs root elongation, but that there might be functional redundancy for *WEE1* at the G2/M transition. Alternatively, if it is accepted that all plants in the wild are under stress, or able to respond rapidly to stress, then reconciling these different observations about *WEE1* may be easier than we think; time will tell.

Equally, if not more puzzling, is the lack of a bona fide CDC25 gene in higher plants that is orthologous to yeast, animal and lower plant CDC25s. In most eukaryotes, CDC25 phosphatase is the lone positive activator that drives a cell into mitosis at the slender G2/M window of opportunity. A small CDC25 in *Arabidopsis* has been cloned that can exhibit phosphatase activity (Landrieu et al. 2004) and is able to induce a small cell size phenotype in fission yeast (Sorrell et al. 2005). Also, dephosphorylation of plant CDK at G2/M was demonstrated through cytokinin treatment in cultured tobacco (*Nicotiana plumbaginifolia*) cells (Zhang et al. 1996, 2005). However, *Arath*;*CDC25* only encodes a catalytic domain and is expressed weakly in most plant tissues (Sorrell et al. 2005). These data, and the knowledge that *Arath*;*CDC25* protein can also exhibit arsenate reductase activity (Bleeker et al. 2006) led Boudolf et al. (2006) to hypothesise that the G2/M transition is regulated firstly through CDKB that phosphorylates an inhibitor of CDKA activity (see below). It is proposed that the phosphorylated inhibitor then releases CDKA to drive cells into mitosis (Fig. 2).

6.3 Negative Regulation of CDKS by the ICKS

A third level of CDK repression in interphase is through interactors (or sometimes called inhibitors) of CDK activity (ICKs). The first ICK was identified in *Arabidopsis* (Wang et al. 1997). There are seven ICKs in *Arabidopsis* although

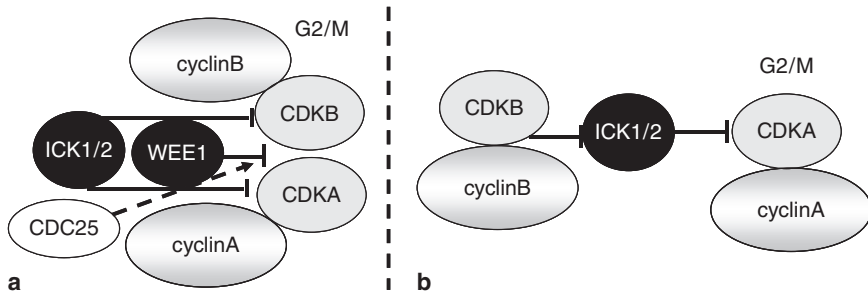


Fig. 2 (a) An orthodox model for G2/M where WEE1 and ICK1/2 repress whilst cdc25 activates the CDKs, (b) a model based on the lack of a bona fide plant CDC25 where CDKB inactivates ICK1/2 that releases CDKA to drive cells into mitosis

2–7 are more commonly referred to as KRP- (Kip-related-) -proteins. *ICK1* can be induced by abscisic acid (Wang et al. 1998) and salt stress (Ruggiero et al. 2004; Wang et al. 2007 for a review of ICKs). Yeast two hybrid protein–protein interaction data show that whilst ICK1 interacts with CDKA;1 it does not interact with B-type CDKs (Wang et al. 2007). This might have repercussions for the Boudolf et al. (2006) model of a CDKB repressing ICK1 repressing CDKA model of releasing cells into mitosis (Fig. 2).

7 Cytokinesis

At telophase of mitosis, two newly partitioned sets of chromosomes are each housed in two new nuclei spaced apart in the mother cell. Cytokinesis in plants occurs when a new cell wall is placed between the newly constituted nuclei. It is a topic that has been extremely well investigated over the past 10 years, and the published literature on plant cytokinesis really merits a PiB chapter of its own. My inclusion of cytokinesis in this chapter will do little justice to the enormous effort that has and is being expended to research it. Nevertheless, not mentioning cytokinesis would be unforgivable.

At cytokinesis, residual microtubules from the mitotic tubular machinery are left at the division plane. This is the phragmoplast which acts as the foundation for the new cell wall. As one might expect, there are cytokinesis-specific genes, one of which is *KNOLLE* that encodes a syntaxin protein (Lauber et al. 1997; Völker et al. 2001); *knolle* mutant embryos have irregular cell patterns and are deficient in the cytokinesis process (elegantly reviewed by Jürgens 2005). An interacting gene is *KEULE* that binds to *KNOLLE* (Asaad et al. 1996). Moreover, there is a complex of proteins comprising SNARE, SYP31 and CDC48 that interact with *KNOLLE* to regulate the process of vesicle fusion at the cell plate. (Heese et al. 2001; Feiler et al. 1995). The so called SNARE-complex also interacts with the AAA AtPase, NSF which all form another part of the protein complex that is essential for

cytokinesis to take place (Rancour et al. 2002). Cell plate formation may be considered as a modified form of vesicle secretion per se (reviewed by Sanderfoot 2007). This in turn seems to rely on the Golgi complex as the provider of vesicles that contribute to cell wall plate formation. The trafficking of these vesicles to the cell plate is under the control of specific motor proteins as mentioned above.

The finishing touch to cytokinins is obviously the new cell wall. In effect, islands of cellulose synthase complex are transported to the outer surface of the plasma membrane, and there they lay down cellulose microfibrils in paths resembling snails criss-crossing an open surface (Burgess 1985). These walls ultimately comprise an interwoven network cellulose and the non-fibrillar pectins and hemicelluloses, together with (hydroxy)proline-rich glycoproteins. In *Arabidopsis*, AtEXT3, a structural glycoprotein, is considered to be part of the scaffold that enables the construction of the primary cell wall (Cannon et al. 2008).

Dynamin-related proteins are large GTPases that are involved in cytokinesis (Praefke and McMahon 2004). Of the 16 DRPs in *Arabidopsis*, three accumulate in the cell plate (Lauber et al. 1997; Otegui et al. 2001). Very recently a *rsw* null mutant of *Arabidopsis* was characterised with a reduced content of cellulose in its cell walls. The mutant phenotype of short and swollen roots is believed to be because of changes in plasma membrane dynamics that inhibit cellulose synthesis (Collings et al. 2008).

8 Conclusions

Clearly, bit by bit, the plant cell cycle is being accounted for on a gene by gene basis, but it does have gaps and riddles. Why so many cyclins, why no CDC25? How exactly does the cell cycle interface with growth, development and extra cellular signalling? The proteomics era offers the possibility of understanding plant cell cycle genes in functional networks of the sort that are beginning to be resolved regulating cytokinesis. The uniqueness of plants with their sessile growth habit will no doubt continue to pose unusual answers to what might seem relatively straightforward questions about the plant cell cycle. In this short review, I have tried to focus on what is new in the plant cell cycle in the past two years (at least to me) but, undoubtedly, there is much more to come.

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