When plant cells decide to divide

Hilde Stals and Dirk Inzé

Progression through the cell cycle is central to cell proliferation and fundamental to the growth and development of all multicellular organisms, including higher plants. The periodic activation of complexes containing cyclins and cyclin-dependent kinases mediates the temporal regulation of the cell-cycle transitions. Here, we highlight recent advances in the molecular controls of the cell cycle in plant cells, with special emphasis on how hormonal signals can modulate the regulation of cyclin-dependent kinases.

Hilde Stals
Dirk Inzé*
Vakgroep Moleculaire Genetica, Dept Plantengenetica, Vlaams Interuniversitair Instituut voor Biotechnologie (VIB), Universiteit Gent, B-9000 Gent, Belgium.
*e-mail: diinz@gengenp.rug.ac.be

The cell cycle is a highly ordered process that results in the formation of two daughter cells and is usually divided into four phases: G1, S (DNA replication), G2 and M (karyo- and cytokinesis) (Fig. 1). Ensuring that each new daughter cell receives a full complement of the hereditary material requires the correct alternation between S phase and M phase. The basic control mechanisms that regulate the progression through the cell cycle are remarkably well conserved through evolution. The main drivers of the cell cycle in yeast, mammals and plants are a class of highly conserved serine/threonine kinases known as the cyclin-dependent kinases (CDKs). Multiple mechanisms have evolved that strictly regulate the CDK activity to maintain the correct temporal ordering of critical cell cycle events, such as DNA replication and spindle assembly.

Here, we review recent advances in our understanding of how the cell-cycle control mechanisms act at the G1–S and G2–M transitions in plants, and we further highlight how hormonal signals are integrated into the cell cycle.

G1 entry and the G1–S transition

At a certain point in the G1 phase, known as START in yeast and as the restriction point in mammals, cells either continue through the cell cycle or stop to differentiate (Fig. 1). In mammals, progression through the restriction point is mediated by D-type cyclins, which also integrate extracellular signals. The continuous presence of growth factors induces the transcription of D-type cyclins and their association with CDK4 or CDK6. The CDK4/6–cyclin-D complexes that are activated via phosphorylation by a CDK-activating kinase (CAK) phosphorylate and inactivate the retinoblastoma protein (RB), thereby activating E2F-controlled genes, which are required for S phase progression. Most of the key players in the RB pathway are conserved throughout the evolution of multicellular organisms, including plants[1], in contrast with yeast and other unicellular organisms, in which no functional homologues of RB have been found to date.

Four classes of D-type cyclins have been identified in plants. Cyclins of the *CycD3* class play a role during S phase entry in response to plant hormones such as cytokinins and brassinosteroids, whereas *CycD2* and *CycD4* are activated earlier in G1 and respond to sugar availability[2–5]. In spite of the extensive list of plant CDKs, no direct equivalents of CDK4/6, the catalytic partners of D-type cyclins in animals, are known in plants. Based on sequence analysis with homologues from other eukaryotes, the family of plant CDKs is divided into five subtypes (A–E)[6]. In G1 phase, only CDKA;1, the homologue of mammalian CDK1, is produced and has been shown to interact with CycA2;1, CycD2;1 and CycD3;1 (Refs 7–10). Moreover, the CDKA;1–CycD3;1 complex of tobacco formed in insect cells can phosphorylate the tobacco RB-related protein *in vitro*[9]. RB-related proteins have been found in plant species such as maize, tobacco, *Arabidopsis*, pea, poplar and *Chenopodium rubrum*[11]. The maize RB protein, Zeama;RB1, and the human RB protein bind all classes of plant D-type cyclins *in vitro*, with the involvement of a conserved N-terminal LxCxE RB-interaction motif[12].

To date, protein inhibitors that modulate the CDK activity have only been identified in *Arabidopsis*, which contains genes for seven CDK inhibitors (ICKs) with distant sequence homology at their C-termini to the CDK-binding or inhibitory domain of $p27^{KIP2}$ (Refs 13,14; L. De Veylder, pers. commun.). The CDKA;1 kinase activity of *Arabidopsis* is inhibited *in vitro* by ICK1 and ICK2, both of which interact with CycD3;1 and CDKA;1 in an *in vitro* binding assay[13,14]. Transgenic *Arabidopsis* plants that overproduce ICK1 and ICK2 have a reduced CDK activity and fewer, but greatly enlarged, cells, showing for the first time the *in vivo* function of a CDK inhibitor *in planta*[15] (L. De Veylder, pers. commun.).

The RB tumour suppressor protein exerts its activity largely by binding the E2F family of DNA-binding transcription factors[16]. E2F sites are found in promoters of multiple plant and animal genes that are involved in cell-cycle progression and DNA replication[17–19]. E2F binds DNA as a heterodimer composed of two structurally related subunits, E2F and its heterodimerization partner (DP) (Fig. 1). The ability of an RB-related protein from maize to bind human and *Drosophila* E2F, and to inhibit the transcriptional activation of human E2F supported the existence of an RB–E2F pathway in plants[12]. The subsequent isolation of E2F homologues from wheat, tobacco, carrot and *Arabidopsis* confirmed this prediction[20–23]. Plant E2Fs exhibit transactivation properties in mammalian and plant cells, and have been shown to interact specifically with the E2F DNA-binding sequences[17,20,24]. Both the DNA-binding and transactivation activity of plant E2Fs required heterodimerization with a human DP protein, which suggested the existence of DP-related proteins in

http://plants.trends.com 1360-1385/01/$ – see front matter PII: S1360-1385(01)02016-7

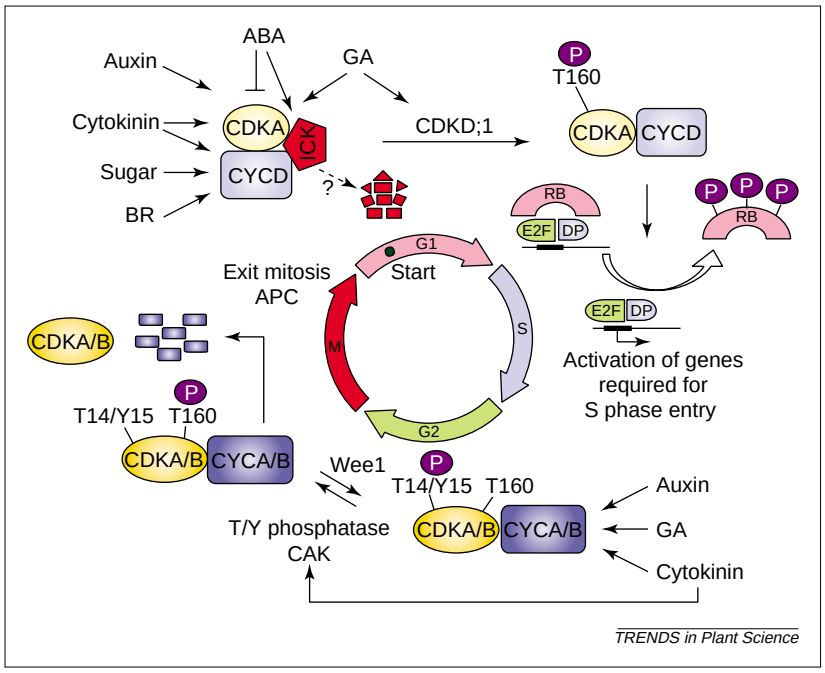

Fig. 1. Model for G1–S and G2–M transitions in plants based on results obtained in plants and on parallels with the mammalian cell-cycle control. During G1, several growth factors, such as auxin, cytokinin, abscisic acid (ABA), gibberellin (GA), brassinosteroids (BR) and sugar regulate the expression of D-type cyclins (CycD) and their catalytic subunit, cyclin-dependent kinase A (CDKA). Activation of the CDKA–CycD complex requires dissociation of the CDK inhibitory protein (ICK), the transcription of which is induced by the stress-responsive hormone ABA and phosphorylation of the Thr160 residue of CDKA by the CDK-activating kinase, CDKD;1, which is upregulated by GA. The active CDKA–CycD complex initiates the phosphorylation of retinoblastoma protein (RB) during late G1 phase, thereby releasing the E2F–DP complex that promotes the transcription necessary for progression into S phase. As mitotic activators auxin, cytokinin and GA also regulate the kinase activity of A- and B-type CDKs by activating the transcription of CDKs and of A- and B-type cyclins. The G2–M transition is associated with an activating Thr160 phosphorylation of CDK by a CDK-activating kinase (CAK) and by dephosphorylation of the inhibitory Tyr phosphorylation that is induced by cytokinin. A ubiquitin-dependent degradation pathway targets B-type cyclins for proteolysis by the anaphase-promoting complex (APC) at the metaphase–anaphase transition, thereby activating the exit from mitosis.

plants as well[20]. This hypothesis was confirmed by the isolation of two distinct *DP*-related genes from wheat and *Arabidopsis*[21,24]. *In vitro* binding assays demonstrated that the formation of E2F–DP complexes in *Arabidopsis* depends on the presence of their heterodimerization domains[21]. The cell-cycle-dependent expression of the plant *E2F* and *DP*-related genes, which are most abundant during early S phase[20–23], further supports their involvement in the regulation of S phase progression in plants.

All these findings strengthen the hypothesis that, during evolution, multicellular organisms as different as plants and animals evolved a similar pathway to control the G1–S transition. This might have originated in a primitive multicellular eukaryote before the divergence of the plant and animal kingdoms. Moreover, this common pathway relies on homologous proteins that are unrelated to those that control the G1–S transition in unicellular organisms such as yeast. In animals, the RB pathway is involved not only in cell-cycle progression but also in the control of programmed cell death. Whether this is also the case for plants is still unknown. Nevertheless, the recent discovery of plant genes encoding prohibitins[25] suggests that there might be a connection between cell-cycle control and cell death in plants as well. Homologues of other animal tumour suppressor genes, such as *p53*, have not been found in plants yet[26].

G2–M transition

Once the cell has duplicated its DNA during the S phase, its next tasks are to generate a mitotic spindle, disassemble the nuclear envelope, condense its chromosomes and align each pair of sister chromatids on the metaphase plate. Expression analysis of five different classes of plant CDKs revealed that the A-type CDKs, like the animal and yeast homologues, are constitutively transcribed. By contrast, the B-type CDKs, which represent a plant-specific gene family, show a cell-cycle-dependent expression pattern, with transcript and protein levels accumulating in G2–M cells[27]. Immunoprecipitation with specific antibodies against A- and B-type CDKs clearly showed histone-H1-phosphorylating activity for CDKA in S, G2 and M phases, and for CDKB during G2–M transition[28–30] (A. Porceddu *et al.*, unpublished). These data suggest that, in contrast with animals and yeast, at least two kinases regulate the G2–M transition in plants (Fig. 1). A potential role for B-type CDKs during entry of mitosis is further supported by the observation that downregulation of B-type CDKs in transgenic plants increases the relative duration of G2 phase (A. Porceddu *et al.*, unpublished).

Limited data are available on the cyclin partners of CDKA or CDKB during the G2–M transition, although both proteins probably bind plant cyclins expressed at the same timepoint[27]. The number of known plant cyclin genes has increased rapidly during the past decade. Completion of the genome-sequencing program of *Arabidopsis* indicated the existence of 27 different cyclins that can be classified as A, B, D and H types by sequence comparison with their mammalian homologues (K. Vandepoele, pers. commun.); the A, B and D types are studied most intensively[27]. The transcript levels of most of the plant D-type cyclins are almost constant during the cell cycle, reminiscent of the animal D-type cyclins[31]. However, two genes for cyclin D homologues from tobacco (*Nicta;CycD2;1* and *Nicta;CycD3;1*) show a mitotic accumulation of their transcripts in synchronized BY-2 cells[32], suggesting that these D-type cyclins are required for entry into or progression through mitosis. Alternatively, the mitotic accumulation of the D-type cyclins could be a BY-2-cell-specific phenomenon as the result of a deregulation of their expression caused by long-term culturing.

Most plant A- and B-type cyclins show a mitotic expression pattern[27], with the exception of *Medsa;CycA2;1* from alfalfa. This cyclin has constitutive transcript and protein levels during the cell cycle, yet its associated kinase activity is biphasic, peaking during S phase and at the G2–M transition[10]. The CDK responsible for both histone-H1-kinase activities is probably CDKA;1, because CycA2;1 interacts with CDKA;1 in a yeast two-hybrid system[10]. The kinase activity associated with B-type

cyclins correlates well with their protein levels, being highest during the G2–M phase transition and disappearing at the exit of mitosis[33]. Similar to their animal and yeast homologues, plant mitotic cyclins contain a destruction box, which targets them for ubiquitin-dependent degradation during mitosis[33,34] (Fig. 1). It has been well established in other systems that CDK activity needs to be switched off during mitotic exit and during G1 to continue through the cell cycle. Overproduction of a non-degradable B-type cyclin in yeast[35] and mammalian systems[36] causes a mitotic arrest. However, no mitotic arrest was observed[37] during the ectopic expression of a non-degradable *clb2* in yeast at modest levels. Similarly, ectopically expressed *Nicta;CycB1;1* in tobacco BY-2 cells did not arrest the cell cycle, suggesting that plants, like yeast, might possess additional mechanisms to inactivate the CDK activity[33].

Full activation of the mitotic CDK activity requires not only cyclin association but also specific phosphorylation–dephosphorylation events (Fig. 1). Dephosphorylation at the inhibitory phosphorylation sites (Thr14 and Tyr15 in human CDK2) by a dual-specificity phosphatase, CDC25, and phosphorylation of a Thr residue within the T-loop region (Thr160 in human CDK2) are additional regulatory mechanisms[38]. Phosphorylation of the Thr160 residue is catalysed by at least two structurally distinct types of CAKs: the trimeric CDK7–CycH–Mat1 complex in metazoans and the single-subunit Cak1 in budding yeast[39]. Fission yeast has both CAK types, with the multisubunit kinase (Mcs6) acting as the Cdc2-activating kinase and the single-subunit kinase (Csk1) as the Mcs6-activating kinase[40].

To date, two plant CAKs have been isolated from rice and *Arabidopsis*, and they have been renamed as CDKD;1 (Ref. 6). *In vitro*, rice CDKD;1 not only phosphorylates human CDK2 and rice CDKA;1 at the Thr residue within their T-loop but also, similarly to the CDK7–CycH–Mat1 complex, phosphorylates the C-terminal domain (CTD) of RNA polymerase II of *Arabidopsis*[41]. Moreover, the rice CDKD;1 kinase is regulated positively by an H-type cyclin, Orysa;CycH;1 (Ref. 42). However, because the transcription of both genes is induced when cells enter S phase, the rice CAK is probably more involved in regulating S phase progression[42,43]. The *CAK1At* of *Arabidopsis*, now designated *Arath;CDKD;1* (Ref. 6), was isolated as a suppressor of the temperature-sensitive *cak* mutant of budding yeast[44]. Although *Arath;CDKD;1* has the closest similarity to *CDK7* from metazoans, a phylogenetic analysis showed that *Arath;CDKD;1* is distinct from *CDK7* and also unrelated to *Cak1* of *Saccharomyces cerevisiae*, suggesting that it might be a novel type of CDK-activating kinase[44]. Moreover, *Arath;CDKD;1* can phosphorylate only human CDK2 and not *Arabidopsis* CTD (Ref. 44); this contrasts with the CAKs of vertebrates and fission yeast, which phosphorylate both CTD and CDKs.

Ectopic expression of *Arath;CDKD;1* in *Arabidopsis* caused an extensive phosphorylation of CDKA;1. Surprisingly, the associated kinase activity of CDKA;1 was reduced without effecting the protein amount[45], indicating that other limiting mechanisms are involved. Thus, plants seem to have two distantly related CAKs (Ref. 6), which suggests a redundancy for the phosphorylation of CDKs. However, these plant CAKs might have distinct functions, acting at different phases of the cell cycle and/or phosphorylating different subtypes of CDKs. An unanswered, but interesting, question is whether CAK of *Arabidopsis* interacts with a cyclin subunit to form an active CAK complex. Identification of the regulatory subunit might help determine the substrate specificity of both plant CAKs. Recently, a putative H-type cyclin has been identified in the *Arabidopsis* genome (K. Vandepoele, pers. commun.).

A WEE1 homologue, which is responsible for the phosphorylation of CDKs at Thr14/Tyr15, has been isolated from maize endosperm and inhibited the $p13^{Suc1}$-adsorbed mitotic CDK activity[46]. In maize endosperm, the M-phase-associated CDK activity decreases at the onset of endoreduplication because of the presence of an inhibitory factor, whereas the S-phase-related kinases are induced[47]. Because *Zeama;WEE1* transcripts are most abundant in actively dividing tissue and accumulate in maize endosperm during the period of endoreduplication[46], this kinase could be one of the factors that inhibit the mitotic CDK activity.

The CDC25 homologue of plants has not been cloned yet and the finalization of the *Arabidopsis* genome-sequencing program showed that *Arabidopsis* does not have a CDC25 homologue[26]. Nevertheless, recombinant CDC25 phosphatase from yeast or *Drosophila* activates the $p13^{Suc1}$-bound CDK fraction of tobacco and alfalfa cells *in vitro*[30,48]. The importance of the Thr/Tyr phosphorylation for the timing of mitosis entry in plant cells is further supported by tobacco transformants that express the yeast *CDC25* gene, in which cells divide at a reduced cell size[49]. Therefore, an unidentified dual-specificity phosphatase could be responsible for the dephosphorylation of the inhibitory Thr/Tyr residues in plants.

Hormone signalling and cell division

Owing to their sessile lifestyle, plants have to respond to local environmental conditions by changing their physiology and redirecting their growth. Signals from the environment include light and pathogen attack, temperature, water, nutrients, touch, and gravity. In addition to local cellular responses, some stimuli are communicated across the plant body by hormones, which consequently play an important role in diverse aspects of plant growth and development. At a cellular level, auxin affects division, expansion and differentiation. Auxin increases both CDKA;1 and mitotic cyclin mRNA levels in roots in conjugation with the induction of cell division[50–52] (Fig. 1).

Although auxin is sufficient to induce the *CDKA;1* expression, it is not in itself enough to stimulate cell division in most cultured cells[48,53]. In tobacco root explants, the catalytic activity of the kinase and the following entry into mitosis is induced only by the addition of cytokinin, stimulating Tyr dephosphorylation of CDKA;1 kinase[48].

Recent studies with *Arabidopsis* have revealed that the ubiquitin proteolysis system plays a central role in the auxin response pathway[54]. The *auxin transport inhibitor resistant 1* (*TIR1*) gene encodes an F-box protein that interacts with plant orthologues of SKP1 (called ASK1 and AKS2) and a cullin protein (Arath;CUL1) to form the ubiquitin protein ligase complex SCF (Ref. 54). How does auxin affect the cell-cycle machinery? One of the effects of auxin is to induce lateral root formation by initiating pericycle cell division from G2 phase, a process that is preceded by increased *CycB1;1* expression. In *tir1-1* mutants (which carry an *Arath;CycB1;1–gus* reporter gene) grown on unsupplemented nutrient medium, reporter-gene expression is lower than that of wild-type seedlings, corresponding to the reduced number of lateral roots that develops in *tir1-1* mutants. These observations indicate that *TIR1* is required directly or indirectly before the expression of *Arath;CycB1;1* in lateral root development[54]. Thus, auxin-promoted pericycle cell division might be achieved by SCF^{TIR1}-facilitated degradation of one or more negative regulators of the cell cycle.

Cytokinins are necessary, in concert with auxin, for cell division at the G1–S and G2–M transitions in a variety of cultured plant cells and *in planta*. When exogenous cytokinin is applied, the expression of *CDKA;1* increases and its kinase activity is induced by promoting the CDKA;1 inhibitory Tyr dephosphorylation at the G2–M transition[48] (Fig. 1). At the G1–S transition, cytokinin regulates cell-cycle progression partly by inducing *CycD3;1* transcription[31] (Fig. 1). Constitutive expression of *CycD3;1* in *Arabidopsis* is sufficient to bypass the need for cytokinins in tissue culture, indicating that cytokinin might promote cell proliferation by inducing this D-type cyclin[4]. *CycD3* transcripts are also upregulated by brassinosteroids (BRs) (Fig. 1), which can substitute for cytokinin in promoting cell proliferation of callus and suspension cultures[3]. Unlike the cytokinin-induced *CycD3* transcription, the BR signal is not mediated by protein phosphorylation but needs protein synthesis[3]. *In planta*, BRs regulate hypocotyl elongation. BR treatment can partly rescue the short-hypocotyl phenotype of the *CDKB1;1*-inducible antisense *Arabidopsis* seedlings when grown in the dark[55]. Furthermore, BRs enhance the *CDKB1;1* expression observed in the hook region of dark-grown seedlings[55]. These results indicate that the *CDKB1;1* gene induced by BRs in darkness could titrate out the *CDKB1;1* antisense mRNA in the inducible antisense transgenic seedlings. However, because BRs cannot fully rescue the short-hypocotyl phenotype of induced antisense *CDKB1;1* transgenics, it is possible that BRs are only part of the regulatory system controlling *CDKB1;1* expression[55].

Upon submergence in water, cell division and cell elongation are accelerated in the intercalary meristems of deep-water rice internodes by gibberellin. This mitogenic hormone initially induces, by an unknown mechanism, A-type CDK and rice CDKD;1 mRNAs at the G1–S transition[43] (Fig. 1). Before the subsequent increase in mitotic B-type cyclin gene expression during late G2, *CycA1;1* and *CDKB1;1* are induced[56] (Fig. 1), which suggests that they might play distinct roles in the regulation of the G2–M transition.

The stress-responsive hormone abscisic acid inhibits cell division in response to adverse environmental cues. This effect might be mediated by the induction of a CDK inhibitor, *ICK1*, which might, together with decreased *CDKA;1* gene expression, result in the observed lower CDK activity[14,52] (Fig. 1).

The receptor mechanism that senses and transmits growth factors in plants is largely unknown. Receptor tyrosine kinases, which play a central role in binding growth factors and thereby regulate several cytoplasmic signal transduction cascades in mammalian cells, do not seem to exist in plants[26]. However, structurally similar receptor kinases that phosphorylate Ser/Thr residues could be their functional equivalents. The best-characterized signal transduction cascade is the mitogen-activated-protein

Acknoledgements
We thank Peter Casteels and Jérôme Joubès for critical reading of the manuscript, Martine De Cock for help preparing it, and Rebecca Verbanck for help with the figures.

Questions for future research

In recent years, considerable progress has been made in understanding the core cell-cycle machinery. The major challenge for future research is to elucidate the mechanisms by which developmental signals and environmental cues communicate with the basic cell-cycle engine.

- What triggers cell division?
- How do plant hormones communicate with the core cell-cycle machinery?
- What causes dividing cells to exit the cell cycle, to elongate and differentiate?
- Which signals convert a mitotic cell cycle into an endoreduplicating cell cycle?
- Which mechanisms are involved in the positioning of the division plane?
- How are cell division and cell growth coupled?

Answering these questions will require a worldwide, multidisciplinary effort. Much progress should be made by combining knowledge on cell-cycle genes (such as cell-cycle phase and cellular specificity of expression, subcellular localization of the proteins, and two-hybrid interactors) with knowledge on signalling pathways that are important for development and interaction with the environment. Two model systems will probably drive future progress: tobacco BY-2 cells, to study the cellular signalling, and *Arabidopsis*, to integrate cell cycle and development. The availability of ever-growing collections of *Arabidopsis* mutants will be of invaluable help. In addition, new collections of conditional mutants, such as temperature-sensitive mutants for growth, will probably contribute to the further elucidation of cell division as an essential process.

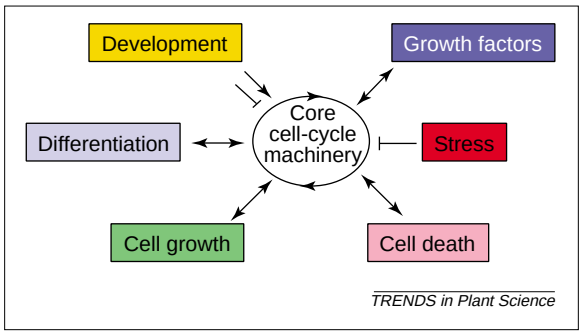

Fig. 2. The major challenge for the future will be the study of the clusters of different signal transduction pathways in plants, by which different processes, such as stress responses, development, cell elongation and differentiation, hormone responses, and cell death are integrated into the core of the cell-cycle machinery and thereby modulate patterns or rate of cell proliferation. Bars indicate inhibition; single-headed arrows indicate activation; double-headed arrows indicate unknown regulatory mechanism.

kinase (MAPK) cascade, which is implicated in cellular activities including proliferation, differentiation, division and death[57]. Although various components of the MAPK cascade have been isolated in plants, no MAPK signal transduction pathway that integrates mitotic stimuli into the core of the plant cell cycle has been established conclusively.

Conclusions

Future challenges lie in unraveling the clusters of different signal transduction pathways in plants by which environmental cues are integrated into the control of the cell cycle, thereby changing the patterns or rate of development. Because the cell cycle is intimately linked to plant development, engineering cell-cycle genes has proved to be a powerful tool to affect plant architecture and important agricultural traits, such as growth rate[58]. Now that the genome sequence of *Arabidopsis* has been finalized and those of other plants are being sequenced, functional genomics and proteomics will be important techniques for determining the global molecular responses that impinge on the cell cycle (Fig. 2). This immense task should ultimately enable us to understand how the core of the cell-cycle machinery is integrated with processes as different as stress responses, development, cell elongation and differentiation, hormone responses and cell death (Fig. 2).

References

1 den Boer, B.G. and Murray, J.A. (2000) Triggering the cell cycle in plants. *Trends Cell Biol.* 10, 245–250
2 De Veylder, L. *et al.* (1999) A new D-type cyclin of *Arabidopsis thaliana* expressed during lateral root primordia formation. *Planta* 208, 453–462
3 Hu, Y. *et al.* (2000) Promotive effect of brassinosteroids on cell division involves a distinct *CycD3*-induction pathway in *Arabidopsis*. *Plant J.* 24, 693–701
4 Riou-Khamlichi, C. *et al.* (1999) Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. *Science* 283, 1541–1544
5 Riou-Khamlichi, C. *et al.* (2000) Sugar control of the plant cell cycle: differential regulation of *Arabidopsis* D-type cyclin gene expression. *Mol. Cell. Biol.* 20, 4513–4521
6 Joubès, J. *et al.* (2000) CDK-related protein kinases in plants. *Plant Mol. Biol.* 43, 607–620
7 Cockcroft, C.E. *et al.* (2000) Cyclin D control of growth rate in plants. *Nature* 405, 575–579
8 Healy, J.M. *et al.* (2001) The *Arabidopsis* D-type cyclins CycD2 and CycD3 both interact *in vivo* with the PSTAIRE cyclin-dependent kinase Cdc2a but are differentially controlled. *J. Biol. Chem.* 276, 7041–7047
9 Nakagami, H. *et al.* (1999) Tobacco retinoblastoma-related protein phosphorylated by a distinct cyclin-dependent kinase complex with Cdc2/cyclin D *in vitro*. *Plant J.* 18, 243–252
10 Roudier, F. *et al.* (2000) Cell cycle function of a *Medicago sativa* A2-type cyclin interacting with a PSTAIRE-type cyclin-dependent kinase and a retinoblastoma protein. *Plant J.* 23, 73–83
11 Durfee, T. *et al.* (2000) Retinoblastoma-related proteins in plants: homologues or orthologues of their metazoan counterparts? *Plant Mol. Biol.* 43, 635–642
12 Huntley, R. *et al.* (1998) The maize retinoblastoma protein homologue ZmRb-1 is regulated during leaf development and displays conserved interactions with G1/S regulators and plant cyclin D (CycD) proteins. *Plant Mol. Biol.* 37, 155–169
13 Lui, H. *et al.* (2000) The *Arabidopsis* Cdc2a-interacting protein ICK2 is structurally related to ICK1 and is a potent inhibitor of cyclin-dependent kinase activity *in vitro*. *Plant J.* 21, 379–385
14 Wang, H. *et al.* (1998) ICK1, a cyclin-dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and CycD3 and its expression is induced by abscisic acid. *Plant J.* 15, 501–510
15 Wang, H. *et al.* (2000) Expression of the plant cyclin-dependent kinase inhibitor ICK1 affects cell division, plant growth and morphology. *Plant J.* 24, 613–623
16 Harbour, J.W. and Dean, D.C. (2000) The Rb/E2F pathway: expanding roles and emerging paradigms. *Genes Dev.* 14, 2393–2409
17 Chabouté, M.E. *et al.* (2000) Cell cycle regulation of the tobacco ribonucleotide reductase small subunit gene is mediated by E2F-like elements. *Plant Cell* 12, 1987–2000
18 Dyson, N. (1998) The regulation of E2F by pRB-family proteins. *Genes Dev.* 12, 2245–2262
19 Gutiérrez, C. (1998) The retinoblastoma pathway in plant cell cycle and development. *Curr. Opin. Plant Biol.* 1, 492–497
20 Albani, D. *et al.* (2000) DcE2F, a functional plant E2F-like transcriptional activator from *Daucus carota*. *J. Biol. Chem.* 275, 19258–19267
21 Magyar, Z. *et al.* (2000) Characterization of two distinct *DP*-related genes from *Arabidopsis thaliana*. *FEBS Lett.* 486, 79–87
22 Sekine, M. *et al.* (1999) Isolation and characterization of the E2F-like gene in plants. *FEBS Lett.* 460, 117–122
23 Ramírez-Parra, E. *et al.* (1999) The cloning of plant E2F, a retinoblastoma-binding protein, reveals unique and conserved features with animal G(1)/S regulators. *Nucleic Acids Res.* 27, 3527–3533
24 Ramírez-Parra, E. and Gutiérrez, C. (2000) Characterization of wheat DP, a heterodimerization partner of the plant E2F transcription factor which stimulates E2F–DNA binding. *FEBS Lett.* 486, 73–78
25 Nadimpalli, R. *et al.* (2000) Prohibitins, stomatins and plant disease response genes compose a protein superfamily that controls cell proliferation, ion channel regulation, and death. *J. Biol. Chem.* 275, 29579–29586
26 The *Arabidopsis* Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408, 796–815
27 Mironov, V. *et al.* (1999) Cyclin-dependent kinases and cell division in plants – the nexus. *Plant Cell* 11, 509–522
28 Bögre, L. *et al.* (1997) The cdc2Ms kinase is differently regulated in the cytoplasm and the nucleus. *Plant Physiol.* 36, 9–19
29 Magyar, Z. *et al.* (1997) Cell cycle phase specificity of putative cyclin-dependent kinase variants in synchronized alfalfa cells. *Plant Cell* 9, 223–235
30 Mészáros, T. *et al.* (2000) Multiple cyclin-dependent kinase complexes and phosphatases control G2/M progression in alfalfa cells. *Plant Mol. Biol.* 43, 595–605
31 Meijer, M. and Murray, J.A.H. (2000) The role and regulation of D-type cyclins in the plant cell cycle. *Plant Mol. Biol.* 43, 621–633
32 Sorrell, D.A. *et al.* (1999) Distinct cyclin D genes show mitotic accumulation or constant levels of transcripts in tobacco bright yellow-2 cells. *Plant Physiol.* 119, 343–352
33 Criqui, M.C. *et al.* (2000) Cell cycle-dependent proteolysis and ectopic overexpression of cyclin B1 in tobacco BY2 cells. *Plant J.* 24, 763–773
34 Genschik, P. *et al.* (1998) Cell cycle-dependent proteolysis in plants. Identification of the destruction box pathway and metaphase arrest produced by the proteasome inhibitor mg132. *Plant Cell* 10, 2063–2076
35 Ghiara, J.B. *et al.* (1991) A cyclin B homolog in *S. cerevisiae*: chronic activation of the Cdc28 protein kinase by cyclin prevents exit from mitosis. *Cell* 65, 163–174
36 Gallant, P. and Nigg, E.A. (1994) Identification of a novel vertebrate cyclin: cyclin B3 shares properties with both A- and B-type cyclins. *EMBO J.* 13, 595–605
37 Amon, A. *et al.* (1994) Closing the cell cycle circle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cycle. *Cell* 77, 1037–1050
38 Russo, A.A. *et al.* (1996) Structural basis of cyclin-dependent kinase activation by phosphorylation. *Nat. Struct. Biol.* 3, 696–700
39 Kaldis, P. (1999) The cdk-activating kinase (CAK): from yeast to mammals. *Cell. Mol. Life Sci.* 55, 284–296

40 Hermand, D. *et al.* (2001) Specificity of cdk activation *in vivo* by two Caks Mcs6 and Csk1 in fission yeast. *EMBO J.* 20, 82–90
41 Yamaguchi, M. *et al.* (1998) A rice homolog of Cdk7/MO15 phosphorylates both cyclin-dependent protein kinases and the carboxy-terminal domain of RNA polymerase II. *Plant J.* 16, 613–619
42 Yamaguchi, M. *et al.* (2000) Activation of CDK-activating kinase is dependent on interaction with H-type cyclins in plants. *Plant J.* 24, 11–20
43 Lorbiecke, R. and Sauter, M. (1999) Adventitious root growth and cell-cycle induction in deepwater rice. *Plant Physiol.* 119, 21–30
44 Umeda, M. *et al.* (1998) A distinct cyclin-dependent kinase-activating kinase of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 95, 5021–5026
45 Umeda, M. *et al.* (2000) A cyclin-dependent kinase-activating kinase regulates differentiation of root initial cells in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13396–13400
46 Sun, Y. *et al.* (1999) Characterization of maize (*Zea mays* L.) Wee1 and its activity in developing endosperm. *Proc. Natl. Acad. Sci. U. S. A.* 96, 4180–4185
47 Grafi, G. and Larkins, B. (1995) Endoreduplication in maize endosperm: involvement of M phase-promoting factor inhibition and induction of S-phase-related kinases. *Science* 269, 1262–1264
48 Zhang, K. *et al.* (1996) Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of $p34^{cdc2}$-like H1 histone kinase. *Planta* 200, 2–12
49 McKibbin, R.S. *et al.* (1998) Expression of fission yeast *cdc25* alters the frequency of lateral root formation in transgenic tobacco. *Plant Mol. Biol.* 36, 601–612
50 Doerner, P. *et al.* (1996) Control of root growth and development by cyclin expression. *Nature* 380, 520–523
51 Ferreira, P.C. *et al.* (1994) Developmental expression of the Arabidopsis cyclin gene *cyc1At*. *Plant Cell* 6, 1763–1774
52 Hemerly, A.S. *et al.* (1993) *cdc2a* expression in Arabidopsis is linked with competence for cell division. *Plant Cell* 5, 1711–1723
53 Tréhin, C. *et al.* (1998) Cell cycle regulation by plant growth regulators: involvement of auxin and cytokinin in the re-entry of *Petunia* protoplasts into the cell cycle. *Planta* 206, 215–224
54 Gray, W.M. *et al.* (1999) Identification of an SCF ubiquitin-ligase complex required for auxin response in *Arabidopsis thaliana*. *Genes Dev.* 13, 1678–1691
55 Yoshizumi, T. *et al.* (1999) An *Arabidopsis* cell cycle-dependent kinase-related gene, *CDC2b*, plays a role in regulating seedling growth in darkness. *Plant Cell* 11, 1883–1896
56 Fabian, T. *et al.* (2000) The cell cycle genes *cycA1;1* and *cdc2Os-3* are coordinately regulated by gibberellin *in planta*. *Planta* 211, 376–383
57 Roovers, K. and Assoian, R.K. (2000) Integrating the MAP kinase signal into the G1 phase cell cycle machinery. *BioEssays* 22, 818–826
58 den Boer, B.G. and Murray, J.A. (2000) Control of plant growth and development through manipulation of cell-cycle genes. *Curr. Opin. Biotechnol.* 11, 138–145

Functional genomics of plant photosynthesis in the fast lane using *Chlamydomonas reinhardtii*

Rachel M. Dent, Miehie Han, and Krishna K. Niyogi

Oxygenic photosynthesis by algae and plants supports much of life on Earth. Several model organisms are used to study this vital process, but the unicellular green alga *Chlamydomonas reinhardtii* offers significant advantages for the genetic dissection of photosynthesis. Recent experiments with *Chlamydomonas* have substantially advanced our understanding of several aspects of photosynthesis, including chloroplast biogenesis, structure–function relationships in photosynthetic complexes, and environmental regulation. *Chlamydomonas* is therefore the organism of choice for elucidating detailed functions of the hundreds of genes involved in plant photosynthesis.

Rachel M. Dent
Miehie Han
Krishna K. Niyogi*
Dept of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102, USA.
*e-mail: niyogi@nature.berkeley.edu

With the genome sequence of the first photosynthetic eukaryote fully characterized, the path appears set for *Arabidopsis* to dominate the field of plant biology in the next decade. But *Arabidopsis* is not the ideal organism for all fields of plant research, and photosynthesis is one area where other models have major advantages (Table 1). *Chlamydomonas reinhardtii* (Fig. 1), for example, has been used as a model organism in photosynthesis research for >40 years, and the use of this unicellular green alga in biochemical, biophysical and genomic approaches, to the study of photosynthesis and photoprotection has been reviewed by several authors recently[1–7]. This review describes several examples of how molecular genetic studies of *Chlamydomonas* have provided new insights into photosynthesis. We will highlight the comparative merits of *Chlamydomonas* as a model photosynthetic organism and discuss how it can make future contributions to the functional genomics of photosynthesis.

Advantages of *Chlamydomonas* for studying photosynthesis

Chlamydomonas has several attributes that make it an excellent organism for basic genetic studies of plant photosynthesis (Table 1). Its photosynthetic apparatus is closely related to that of vascular plants, and it is also a eukaryote, with photosynthesis genes encoded by both the nuclear and chloroplast genomes. As a unicellular organism, *Chlamydomonas* has the advantages of a microbial lifestyle without the complications of multicellularity. Synchronous or asynchronous cultures of *Chlamydomonas* grow quickly with a doubling time of less than ten hours, and the cells behave homogeneously in terms of physiological and biochemical characteristics. Because *Chlamydomonas* is haploid and has a controlled sexual cycle with the possibility of tetrad analysis (Fig. 2), it is an excellent genetic model.

http://plants.trends.com 1360-1385/01/$ – see front matter PII: S1360-1385(01)02018-0