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Cyclin-Dependent Kinases and Cell Division in Plants— The Nexus

Vladimir Mironov,[a,b] Lieven De Veylder,[a] Marc Van Montagu,[a] and Dirk Inzé[a,b,c,1]

[a]Laboratorium voor Genetica, Departement Plantenggenetica, Vlaams Interuniversitair Instituut voor Biotechnologie, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium
[b]CropDesign N.V., Technologiepark 3, B-9052 Zwijnaarde, Belgium
[c]Laboratoire Associé de l'Institut National de la Recherche Agronomique (France), Universiteit Gent, B-9000 Gent, Belgium

INTRODUCTION

Cell division is one of the most conspicuous features of life, and thus several elements of the control of cell division are common to both prokaryotes and eukaryotes (Amon, 1998; Leatherwood, 1998). The degree of evolutionary conservation is especially striking among eukaryotes, where progression through the successive phases of the cell cycle (S, G2, M, and G1) in species as diverse as yeast and humans is driven by a common class of heterodimeric serine/threonine protein kinases. These kinases consist of a catalytic subunit, termed cyclin-dependent kinase (CDK), and an activating subunit, cyclin (reviewed in Nigg, 1995).

The first indication that this commonality might extend to the plant kingdom came with the identification of a plant protein immunologically related to the CDKs (John et al., 1989), a finding that was followed by the cloning of a cDNA fragment encoding a CDK-like protein from pea (Feiler and Jacobs, 1990). Subsequently, it became clear that several putative CDKs and cyclins are present in each plant species and that at least some of them are pertinent to our understanding of cell division control (Hemerly et al., 1995; Doerner et al., 1996).

Several questions nevertheless remain to be addressed. For example, which putative cyclins and CDKs are involved in cell division control in plants? What are their particular functions? How is their activity regulated? Here, we focus on molecular control of the cell cycle in higher plants and do not deal with the developmental and environmental control of cell division. For more information on these latter issues, the reader is referred elsewhere (Francis et al., 1998).

DIVERSITY OF CDKs AND CYCLINS IN PLANTS

Intensive cloning efforts over the past 7 years have identified a large number of CDK-like proteins (referred to as CDKs hereafter) in diverse plant species, among which at least five types can be distinguished on the basis of their sequences (see Segers et al., 1997; summarized in Table 1). The best-characterized plant CDKs belong to the A-type class. This class comprises kinases most closely related to the prototypical CDKs, yeast cdc2/CDC28 and animal CDK1 and CDK2, which share the conserved PSTAIRE motif in the cyclin binding domain. In addition to this large group of CDKs, several non-PSTAIRE CDKs have been described in plants. Some of them cannot be clearly affiliated with any other class of CDKs on the basis of sequence similarity. The situation may be even more complex in plant species that possess more than one representative of a given type of CDK, although it should be noted that not all plant species appear to possess all types of CDKs. For example, no homologs of the rice R2 kinase could be detected in Arabidopsis (Yamaguchi et al., 1998). Currently, only A and B classes of CDKs are well defined; other classes are represented only by one or two known members whose distribution in the plant kingdom remains unclear.

A similar situation exists with the cyclins. Numerous cDNAs encoding putative cyclins (referred to as cyclins hereafter) have been identified in a diverse range of plant species (for a compilation, see Renaudin et al., 1996). Arabidopsis alone possesses at least 15 cyclins. Analysis of the deduced peptide sequences in the conserved "cyclin box" has enabled the classification of these cyclins into nine groups: A1, A2, A3, B1, B2, D1, D2, D3, and D4, with the lettering scheme reflecting their similarities with the mammalian cyclins A, B, and D (Table 2; Renaudin et al., 1996; De Veylder et al., 1999). More recently, cyclins with similarity to mammalian cyclin C have been identified in rice and Arabidopsis, thus adding even greater complexity to efforts to classify plant cyclins.

The classification scheme described above, although helpful, does not necessarily reflect the functional properties of the cyclins. In particular, cyclins from groups A2, B1, D2, and D3 may comprise functionally distinct members, as judged by their subcellular localization and expression patterns (see below). In due course, the completion of the

[1]To whom correspondence should be addressed. E-mail diinz@gengenp.rug.ac.be; fax 32-9-2645349.

Table 1. Classification of CDKs in Plants

Class	Cyclin Binding Motif	Typical Phase Dependence[a]	Members Discussed	Closest Mammalian Homolog	Comments
A-type	PSTAIRE	Nonspecific	CDC2aAt CDC2aMs CDC2bMs CDC2aZm	CDK1, CDK2	Many complement yeast cdc2/CDC28 mutants; expressed in cycling cells and cells showing competence for division; high kinase activity in S, G2, and M
B-type	PPTALRE PPTTLRE	S/G2 G2/M	CDC2bAt CDC2fMs	Unknown Unknown	Do not complement yeast cdc2/CDC28 mutants; maximum kinase activity in M; expressed typically in dividing cells
Nonclassified CDKs	NFTALRE	G1/S?[b]	R2	CDK7 (CAK)	Phosphorylates CDKs and/or the large subunit of RNA polymerase II; complements *civ1/cak1* mutant in budding yeast
	PITAIRE	Nonspecific	CDC2cMs	CHED kinase?	
	SPTAIRE	Nonspecific	CDC2eMs	CDK8?	

[a]At the transcriptional level.
[b]Question marks denote uncertainty.

Arabidopsis genome sequencing project will provide definitive answers to questions regarding the profusion and diversity of plant CDKs and cyclins.

WHICH PLANT CDKs AND CYCLINS ARE AT WORK IN THE CELL CYCLE?

Lately, it has become increasingly clear that certain CDKs and cyclins in yeast and animals have nothing to do with cell division control. Thus, the time is ripe to ask the question, which of the many identified plant CDKs and cyclins are actually involved in regulating the cell cycle? There is now an extensive, albeit mainly circumstantial, body of evidence that at least some plant CDKs and their associated proteins function in cell cycle control. One of the strongest arguments is the ability of many of these proteins to substitute the functions of their yeast and animal homologs (Tables 1 and 2; Hata et al., 1991; Hemerly et al., 1992; Renaudin et al., 1994; Dahl et al., 1995; Meskiene et al., 1995; Setiady et al., 1995; Soni et al., 1995; Day et al., 1996; Ito et al., 1997; Sundaresan and Colasanti, 1998). Although these data might appear to be conclusive, it should be noted that animal B and C cyclins are known to complement G1 cyclin deficiency in yeast despite the fact that there is no indication that they play a role in G1 progression (in the case of cyclin B) or any aspect of cell division (cyclin C) in animals. The consistently observed correlation between cell division and the expression patterns of many plant cyclins and CDKs is, similarly, both supportive and circumstantial.

More compelling data—accelerated progression through mitosis, including a rapid disintegration of the preprophase band (PPB), nuclear envelope breakdown, and chromosome condensation—have been obtained upon injection of active CDK complexes from metaphase plant cells into *Tradescantia* stamen hair cells (Hush et al., 1996). The composition of the complexes used in these studies, however, is unknown.

Currently, experiments in plants support relevance for cell division control only for CDC2aAt, CDC2bAt, and CYCB1;1 and by extrapolation for their orthologs from other species. Hemerly et al. (1995) have demonstrated that downregulation of CDC2aAt activity in plants is sufficient to compromise the rate of cell proliferation and other aspects of cell division, such as the orientation of cell division planes and cell size control. Because such downregulation does not affect the relative duration of G1 and G2, CDC2aAt probably functions in both the G1-to-S and G2-to-M transitions. In a similar way, we have shown that downregulation of B-type CDKs in transgenic plants lengthens the relative duration of G2, thus implicating these kinases in the progression through G2 (V. Mironov, A. Porceddu, J.-P. Reichheld, and D. Inzé, unpublished results). On the other hand, Doerner et al. (1996) have shown that cell proliferation in Arabidopsis roots can be boosted by ectopic expression of the CYCB1;1 cyclin. This work demonstrates that CYCB1;1 might be a limiting factor for cell division in Arabidopsis, although the phase of the cell cycle at which it operates was not identified (Doerner et al., 1996).

There is no evidence for cell cycle functions of PITAIRE or SPTAIRE CDKs. Moreover, the expression pattern of an Arabidopsis PITAIRE kinase closely related to CDC2cMs argues

against the involvement of this group of CDKs in cell division control because no expression associated with actively dividing cells was detected by in situ hybridization (V. Mironov, R.M. de Pinho Barroco, and D. Inzé, unpublished results).

HOW DOES CDK ACTIVITY CHANGE THROUGH THE CELL CYCLE?

Routinely, CDK activity is assessed by histone H1 phosphorylation, and substantial biochemical evidence for the presence of CDK activity in diverse plant cells has been generated by using histone H1 as a substrate for CDK complexes purified by $p13^{suc1}$ and $p9^{CKS1Hs}$ affinity selection (Jacobs, 1995). However, these data are mainly inconclusive and difficult to interpret because the composition of the complexes is in the best case guesswork, given the ability of a number of distinct kinases, including A- and B-type CDKs, to bind $p13^{suc1}$ and $p9^{CKS1Hs}$. In only a few instances has CDK activity been traced down to a specific CDK or cyclin that has previously been identified by cloning. In particular, histone H1 kinase activity specifically associated with A-type CDKs has been analyzed in partially synchronized suspension cells of alfalfa (Bögre et al., 1997; Magyar et al., 1997), Arabidopsis, and tobacco (J.-P. Reichheld and D. Inzé, unpublished results). Most of these data consistently demonstrate high kinase activity in S, G2, and M phases, with a pronounced recession in G1.

By contrast, the activity of B-type kinases is prominently linked to mitosis. We have used specific antibodies against CDC2bAt to immunoprecipitate the histone H1 kinase activity associated with B-type CDKs in partially synchronized

Table 2. Classification of Cyclins in Plants

Class	Typical Phase Dependence[a]	Members Discussed[b]	Original Name	Comments
A1	S/G2/M	Zeama;*CYCA1;1* Nicta;*CYCA1;1*	*cycIIZm* *ntcyc25*	Zeama;*CYCA1;1* triggers frog oocyte maturation; Nicta;*CYCA1;1* rescues G1 cyclin deficiency in yeast
A2	S/G2/M	Nicta;*CYCA2;1* Medsa;*CYCA2;1*[c]	*ntcyc27* *cycMs3*	Medsa;*CYCA2;1* expression suppresses the α-pheromone–induced cell cycle arrest in yeast; Medsa;*CYCA2;1* and Nicta;*CYCA2;1* complement G1 cyclin deficiency in yeast
A3	S/early G2	Catro;*CYCA3;1*	*CYS*	Catro;*CYCA3;1* rescues G1 cyclin deficiency in yeast
B1	G2/M	Arath;*CYCB1;1* Arath;*CYCB1;2* Catro;*CYCB1;1* Nicta;*CYCB1;2* Zeama;*CYCB1;1* Zeama;*CYCB1;2* Glyma;*CYCB1;1*	*cyc1At* *cyc1bAt* *CYM* *ntcyc29* *cycIaZm* *cycIbZm* *S13-6*	Arath;*CYCB1;1*, Zeama;*CYCB1;1*, Zeama;*CYCB1;2*, and Glyma;*CYCB1;2* trigger frog oocyte maturation; Arath;*CYCB1;2*, Catro;*CYCB1;1*, and Nicta;*CYCB1;2* rescue G1 cyclin deficiency in yeast
B2	G2/M	Arath;*CYCB2;2* Zeama;*CYCB2;1* Medsa;*CYCB2;2*	*cyc2bAt* *cycIIIZm* *cycMs2*	Zeama;*CYCB2;1* triggers oocyte maturation; Medsa;*CYCB2;2*-immunoprecipitated kinase activity is maximal in G2
D1	Unknown	Arath;*CYCD1;1*	*cyclin δ1*	Rescues G1 deficiency in yeast; associates with CDC2aAt in the two-hybrid system
D2	Nonspecific	Arath;*CYCD2;1* Nicta;*CYCD2;1*	*cyclin δ2*	Arath;*CYCD2;1* rescues G1 deficiency in yeast; expression sucrose inducible; Nicta;*CYCD2;1* transcript peaks during M
D3	Nonspecific	Arath;*CYCD3;1* Medsa;*CYCD3;1* Nicta;*CYCD3;1* Nicta;*CYCD3;2*	*cyclin δ3* *cycMs4*	Arath;*CYCD3;1* and Medsa;*CYCD3;1* rescue G1 deficiency in yeast, expressed in only a subset of proliferating cells; Arath;*CYCD3;1* cytokinin inducible interacts with Rb and ICK1; Nicta;*CYCD3;1* transcript peaks during M
D4	Unknown	Arath;*CYCD4;1*		Expression sucrose inducible; expressed during lateral root primordia formation

[a] At the transcriptional level.
[b] Nomenclature according to Renaudin et al. (1996).
[c] Expressed in a nonspecific manner.

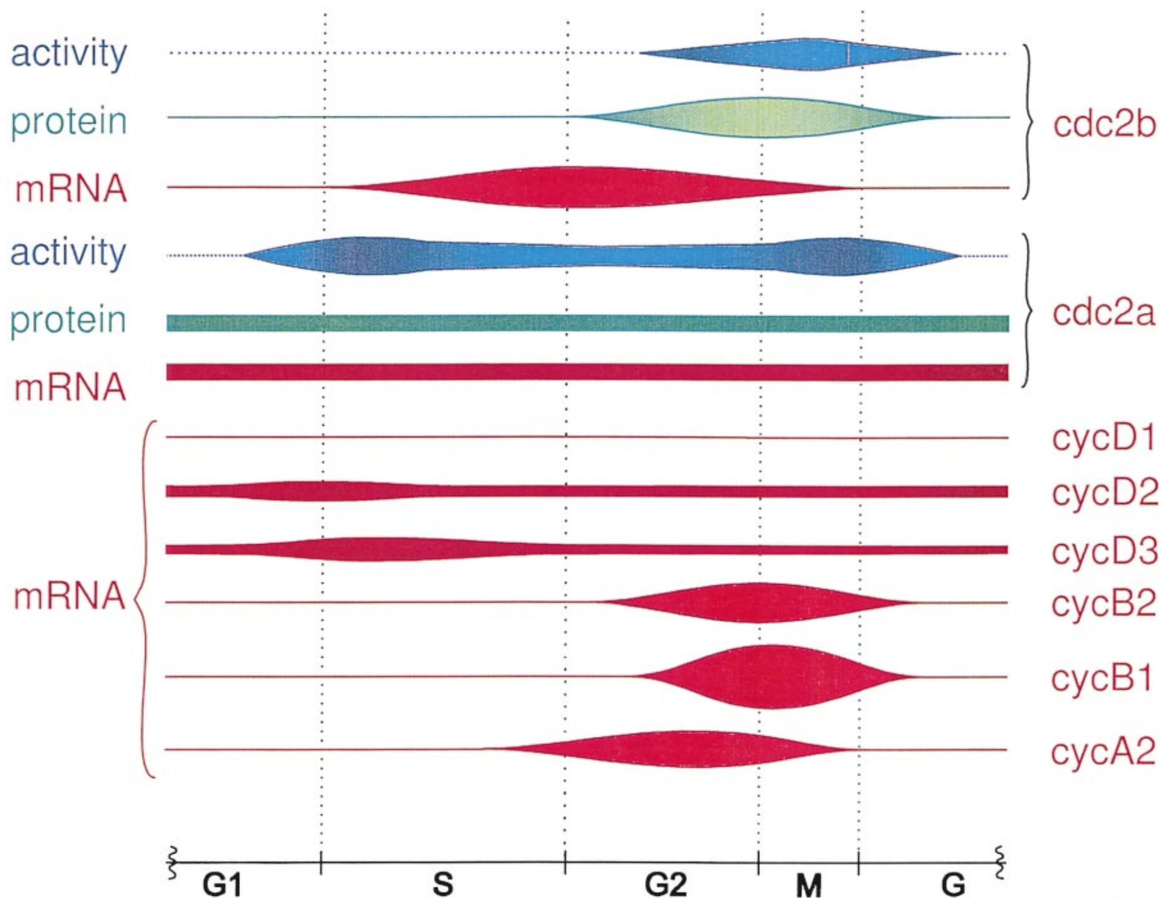

Figure 1. Control of Cell Cycle Genes in Arabidopsis.

Expression of Arabidopsis cyclins, CDKs, and CDK activities (using histone H1 as substrate) over the course of the cell cycle. The thickness of the filled areas qualitatively reflects the level of mRNA, protein, or activity, as indicated.

suspension cells and have found that the activity associated with CDC2bAt in Arabidopsis and the cognate protein in tobacco peak in the early M phase (J.-P. Reichheld and D. Inzé, unpublished results). Similarly, the activity of CDC2fMs sharply peaks in mitosis in partially synchronized alfalfa cells, albeit somewhat later (Magyar et al., 1997). The activity profiles of A- and B-type CDKs are illustrated in Figure 1.

It is still to be seen which cyclins contribute to all of these activities. Currently, the information is limited to the demonstration that histone H1 kinase activity peaks in G2 in complexes immunoprecipitated with antibodies against the alfalfa cyclin CYCB2;2 (Magyar et al., 1997).

There are two indications that biochemically distinct histone H1 kinases, characterized by their inability to bind to an affinity p13^{suc1} matrix, may be activated during DNA replication in plant cells. Indeed, a histone H1 kinase isolated from endoreduplicating maize endosperm cells by virtue of its binding to the human E2F and adenovirus E1A proteins (Grafi and Larkins, 1995) proves to be almost absent from mitotically cycling endosperm cells. This kinase cross-reacts with an antibody against the A-type maize CDK CDC2aZm, but it does not bind p13^{suc1}. In addition, anti–human cyclin A antibodies precipitate a histone H1 kinase (of unknown identity) from alfalfa cells very early in S phase, a kinase that is also not recoverable by p13^{suc1} affinity (Magyar et al., 1993).

No G1-specific CDK activities have been described in plants. In mammals, the G1 kinases consist of CDK4 or CDK6 associated with the D cyclins (Pines, 1996a). Biochemically, these CDKs differ considerably from the other CDKs in that they do not bind p13^{suc1}, and histone H1 is a very poor substrate. Instead, the retinoblastoma protein (pRB), a key regulator of the G1 transition, is the preferred substrate. The cloning of maize cDNAs coding for pRB-like

proteins (reviewed in Gutierrez, 1998) may provide the necessary tool for the detection of G1-specific CDK activities in plants.

WHAT DO WE KNOW ABOUT THE MOLECULAR MECHANISMS OF REGULATION?

In yeast and animals, CDK activity is regulated at several levels, including expression, differential subcellular localization, phosphorylation, proteolysis, and interaction with regulatory proteins. Below, we summarize our current knowledge of these events in plants.

Expression of CDKs and Cyclins

The expression of plant CDKs and cyclins has been studied rather extensively at the level of transcript accumulation. As a result, we know now that some plant CDKs cycle, as do many plant cyclins (Tables 1 and 2 and Figure 1). In particular, all the B-type kinases analyzed so far accumulate transcripts preferentially either in S and G2 or in G2 and M phases (Fobert et al., 1994, 1996; Segers et al., 1996; Magyar et al., 1997; Umeda et al., 1999). In contrast, cell cycle phase–independent expression is typical of the majority of the plant A-type CDKs (Martinez et al., 1992; Hemerly et al., 1993; Magyar et al., 1993, 1997; Fobert et al., 1996; Segers et al., 1996; Setiady et al., 1996; Sauter, 1997; Umeda et al., 1999).

The expression profiles of plant CDKs other than A or B type have drawn much less attention. The transcript of the rice CDK R2 is more abundant in G1 and S in partially synchronized rice suspension cells (Sauter, 1997) but is rather constant in rice root meristems (Umeda et al., 1999). The expression of CDC2cMs and CDC2eMs in partially synchronized alfalfa suspension cells remains constant throughout the cell cycle (Magyar et al., 1997).

As it is in animals, the phase-dependent expression of A- and B-type cyclins in plants is under transcriptional control. Moreover, there seems to be a fair degree of correlation between the temporal expression pattern and the cyclin class as defined by primary structure (Table 2; Fobert et al., 1994; Kouchi et al., 1995; Meskiene et al., 1995; Setiady et al., 1995; Reichheld et al., 1996; Segers et al., 1996; Shaul et al., 1996; Ito et al., 1997; Sauter, 1997; Lorbiecke and Sauter, 1999). Interestingly, the cyclin CYCA2;1 from alfalfa, related to the A2 group, is nevertheless expressed uniformly throughout the cell cycle and has consequently been proposed to play a role in G1 (Meskiene et al., 1995). In terms of the mechanisms of the G1 phase transition in plants, it is important to find out whether functional homologs of Medsa;CYCA2;1 are present in other species.

The majority of D-type cyclins in both plants and animals manifest fairly constant expression levels throughout the cell cycle (Dahl et al., 1995; Soni et al., 1995; Doonan, 1998). Plant D cyclins, by analogy with their animal homologs, have been proposed to control the G1 progression in response to growth factors and nutrients (Dahl et al., 1995; Soni et al., 1995). Unexpectedly, cyclins CYCD2;1 and CYCD3;1 from tobacco are found to be expressed predominantly during the G-to-M transition (Sorrell et al., 1999), suggesting that D-type cyclins in plants may also be involved in mitotic events.

Relatively little is known regarding the degree to which the protein levels of plant cell cycle genes follow the transcriptional patterns described above. The protein levels of A-type CDKs are rather stable throughout the cell cycle (Bögre et al., 1997; Magyar et al., 1997; Mews et al., 1997; J.-P. Reichheld and D. Inzé, unpublished results). The protein levels of B-type CDKs clearly peak in M phase (Magyar et al., 1997; Umeda et al., 1999; J.-P. Reichheld and D. Inzé, unpublished results).

The only relevant information regarding expression of

Table 3. Intracellular Location of Plant CDKs and Cyclins[a]

Location	A-Type CDKs	CYCA1;1	CYCB1;1	CYCB1;2	CYCB2;1
Interphase cytoplasm	±	+	±	+	−
Interphase nuclei	+	±	+	±	+
Prophase nuclei	+	+	+	+	+
Preprophase band	+	+	−	+	−
Mitotic spindle	+	+	−	+	−
Condensing chromosomes	−	−	−	+	−
Nuclear envelope	−	−	−	+	−
Phragmoplast	+	+	−	+	+
Interphase cortical microtubules	−	+	−	−	−

[a](+), strong labeling; (±), weak labeling; (−), undetectable labeling.

plant cyclins is provided by Mews et al. (1997), who used indirect immunofluorescence to localize four mitotic cyclins in the A1, B1, and B2 groups in maize root tip cells (Table 3). Because the signals obtained through immunolocalization may reflect epitope accessibility rather than actual protein levels, data of this kind should be interpreted with caution. Nevertheless, the results seem to confirm the prevalence of the cyclins in G2 and M and further suggest their persistence (with the exception of CYCB1;2) well into telophase. In this regard, it is worth noting that cyclins with specific functions in the completion of mitosis have recently been identified in yeast (Aerne et al., 1998).

Subcellular Localization

A steadily accumulating body of evidence points to the control of subcellular localization of a number of essential proteins, particularly CDC2, cyclin B, cyclin D, CDC25, and CDC6, as an important mechanism of cell cycle control in eukaryotes (Pines, 1999). In plants, this aspect of regulation has been addressed only for A-type CDKs and four mitotic cyclins (Table 3). A-type CDKs, when assayed by indirect immunofluorescence, are predominantly found in the interphase and early prophase nucleus in maize, alfalfa, and Arabidopsis, and to a lesser extent in the cytoplasm (Colasanti et al., 1993; Mews et al., 1996, 1997; Bögre et al., 1997; Stals et al., 1997).

During mitosis, A-type CDKs have been found in association with a number of cytoskeletal structures, such as the PPB, spindle, and phragmoplast. They also transiently interact with the chromosomes at the metaphase–anaphase transition in alfalfa (Stals et al., 1997) but apparently not in maize (Mews et al., 1997). The cytoplasmic labeling progressively declines as the cells of maize root tips exit the mitotic cycle and differentiate. However, the cognate proteins persist in the nuclei through all the developmental zones, including in differentiated cells (Mews et al., 1996). This observation may indicate that nuclear localization renders A-type plant CDKs less susceptible to proteolysis. However, Bögre et al. (1997) have observed that comparable amounts of A-type CDKs are present in the cytoplasmic and nuclear fractions of alfalfa cells in S phase, whereas the proteins are detectable by immunofluorescence only in the nucleus of the same cells. This observation suggests that epitope accessibility of plant CDKs may be influenced by subcellular localization.

The pioneering work of Mews et al. (1997) provides the first piece of data on the subcellular localization of plant cyclins (Table 3). All four cyclins display unique and dynamic patterns of localization, demonstrating that the functions of the numerous plant cyclins are not redundant. Particularly striking is the difference between the two B1 cyclins: whereas CYCB1;1, like CDC2Zm, is predominantly nuclear, CYCB1;2 localization closely resembles that of human cyclin B1 in that this cyclin is relocated to the nucleus in prophase and degraded in anaphase. Nuclear relocation in prophase has also been observed for CYCA1;1, which is at odds with the nuclear localization of animal cyclin A. These results clearly show that the functions of plant cyclins cannot be deduced from sequence similarity with their animal counterparts.

Formation of CDK/Cyclin Complexes

Whereas our knowledge of the expression of CDKs and cyclins in plants is already quite substantial, disappointingly little functional data exist regarding the CDK/cyclin complexes. First of all, we do not know whether plant CDKs are dependent on cyclins. Computer-assisted modeling of the three-dimensional structure of CDC2aAt (R. Abagyan, unpublished data) based on coordinates of the human CDK2 model (De Bondt et al., 1993) suggests that the plant kinase should be as cyclin dependent as the human enzyme. The only supporting experimental evidence, however, is circumstantial: on the one hand, Bögre et al. (1997) found that protein fractions from alfalfa extracts corresponding to monomeric CDKs are essentially devoid of kinase activity, as measured by histone H1 phosphorylation; on the other hand, alfalfa protein complexes immunoprecipitated with antibodies against the human cyclin A or alfalfa cyclin CYCB2;2 exhibit appropriate histone H1 kinase activity (Magyar et al., 1993, 1997).

Not a single active CDK/cyclin complex has been reliably identified in plants. The results of immunolocalization of CDC2Zm and mitotic cyclins in maize suggest several possible combinations (see Table 3), but these data fall short of proof. Two approaches pursued recently in our laboratory are beginning to shed light on the CDK/cyclin complexes of Arabidopsis. First, we have identified a number of proteins capable of interacting with CDC2aAt by using the two-hybrid system, including CYCD1;1 (De Veylder et al., 1997a) and CYCD4;1 (De Veylder et al., 1999). Nevertheless, it still has to be proven that such CDC2aAt/CYCD complexes are actually formed and active in plant cells. Indeed, some of the complexes formed by animal CDKs and cyclins, in particular complexes of cyclin D with CDK2 and CDK5, are known to be inactive (Ewen et al., 1993; Xiong et al., 1997).

Second, a procedure has been developed in our laboratory to purify active kinase complexes from Arabidopsis cells that contain selectively either CDC2aAt or CDC2bAt, whereby CYCB1;1 and CYCB2;2 were found to copurify preferentially with CDC2bAt and CDC2aAt, respectively (H. Stals and P. Casteels, unpublished data). Many more complexes will soon be characterized, but given the plethora of cyclins in plants, it may take some time to achieve a comprehensive overview of the system. The persistence of "orphan" cyclins in the more thoroughly characterized mammalian systems would seem to substantiate this caveat (Pines, 1996a).

Interaction with Other Cell Cycle Regulators

Several noncyclin proteins have been found in complexes with CDKs. In particular, the family of evolutionarily conserved CKS proteins (for cyclin-dependent kinase subunit) is required for progression through the cell cycle in yeast and vertebrates, although the molecular mechanisms by which these proteins act remain elusive. Crystallographic and biochemical analyses suggest that CKS proteins act as docking factors for positive and negative regulators of CDKs (Pines, 1996b).

A plant CKS homolog, CKS1At, has been isolated through the use of a two-hybrid system using CDC2aAt as bait (De Veylder et al., 1997b). The *CKS1At* gene is functional in yeast, and its gene product associates with both A- and B-type Arabidopsis CDKs in vivo (in yeast) and in vitro. In situ hybridization analysis (Jacqmard et al., 1999) further reveals that *CKS1At*, together with *CDC2aAt* and *CDC2bAt*, is strongly transcribed in actively dividing tissues, suggesting that these proteins may also interact in plants. The presence of *CKS1At* expression in a number of polyploid tissues where *CDC2aAt* and *CDC2bAt* transcripts are present at very low levels or are absent (Jacqmard et al., 1999) indicates that CKS1At may play a role in the endocycle. It is conceivable that CKS1At is required for the functioning of a yet to be identified CDK of Arabidopsis, presumably one that is involved in the process of endoreduplication.

Much attention has been focused of late on a group of proteins in yeast and animals known as CDK inhibitors (CKIs). These proteins inhibit cell cycle progression through their association with CDK complexes (Nakayama and Nakayama, 1998). A first plant gene (*ICK1*) with limited sequence similarity to mammalian CKIs was isolated by using a two-hybrid system with the CDC2aAt protein as bait (Wang et al., 1997). Two additional putative plant CKIs have since been isolated in a similar way in our laboratory. Remarkably, all these proteins show only modest sequence similarity to the human $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ inhibitors. This similarity is restricted to a stretch of 30 amino acids located at the C terminus, which has been found crucial for the interaction of ICK1 with CDC2aAt and also CYCD3;1 (Wang et al., 1998). The remainder of the plant protein sequences has no similarity to any other protein in the public databases. Despite that, the results of Wang et al. (1998) show that the region adjacent to the conserved C terminus is, as in the animal counterparts, involved in the interaction with the cyclin (CYCD3;1 in this case). Recombinant ICK1 at nanomolar concentrations inhibits 80% of the total CDK activity (measured with the histone H1 kinase assay) recovered from Arabidopsis extracts by using $p13^{suc1}$ affinity selection (Wang et al., 1997). The failure to inhibit CDK activity completely is most probably due to the specificity of ICK1 for A-type CDKs (L. De Veylder and D. Inzé, unpublished results). Transcriptional induction of *ICK1* by abscisic acid suggests that ICK1 may mediate the cytostatic effect of abscisic acid in plants (Wang et al., 1998).

The in vivo function of the plant CKI-like proteins has still to be determined. The current experimental evidence indicating that CKIs may be deployed in plant cell cycle control is limited to two circumstantial observations. Grafi and Larkins (1995) have shown that cells of maize endosperm undergoing endoreduplication contain an unidentified active inhibitor of the histone H1 kinase activity of mitotically dividing endosperm cells. Bögre et al. (1997) analyzed histone H1 kinase activity of nuclear and cytoplasmic CDKs purified from synchronized alfalfa cells either by immunoprecipitation (A-type CDKs) or by $p13^{suc1}$ binding. The observed differences in the activity profiles suggest the presence of a thermolabile inhibitor, predominantly cytoplasmic, with higher affinity for nuclear S phase CDKs. Although in the absence of some essential controls this interpretation cannot be regarded as definitive, the observed phenomenon provides a promising assay for the biochemical identification of the presumed inhibitor.

CDK Phosphorylation

Considerable progress has been achieved lately in the analysis of the post-translational regulation of CDK/cyclin complexes in plants. Zhang et al. (1996) presented the first direct evidence for the phosphorylation of CDKs as a control mechanism in plants. Tobacco pith parenchyma and *Nicotiana plumbaginifolia* suspension-cultured cells, arrested in G2 by the absence of cytokinin, contain CDK complexes with both reduced kinase activity and high phosphotyrosine content. Resumption of the cell cycle upon addition of cytokinin, however, results in tyrosine dephosphorylation and kinase reactivation. The in vitro treatment of the complexes from cytokinin-depleted cells with the yeast cdc25 phosphatase, highly specific for the Tyr15 of CDKs, similarly leads to their dephosphorylation and activation, implicating the Tyr15 residue as the most probable target of the inhibitory phosphorylation.

Given that Tyr15 is almost universally conserved in plant CDKs, this type of phosphorylation-dependent regulation might well prove to be common in plants. Moreover, the requirement for cytokinin in *N. plumbaginifolia* cells can be completely alleviated by expression of the *cdc25* gene from fission yeast (John, 1998), thus suggesting the triggering of Tyr15 dephosphorylation as the only essential function of cytokinins in the plant cell cycle. The *cdc25* gene has also been expressed in transgenic tobacco plants and in cultured roots of tobacco, and in both cases the cells were found to divide at a reduced size (Bell et al., 1993; McKibbin et al., 1998). This observation further supports the importance of Tyr15 phosphorylation in the timing of mitosis in plants. Tyr15 phosphorylation also has been recently implicated in water stress responses in wheat (Schuppler et al., 1998). The identity of the CDK(s) subjected to inhibitory phosphorylation in all these cases, however, remains unknown.

The question of the function of Tyr15 phosphorylation has been approached from a different angle by Hemerly et al. (1995), who produced transgenic Arabidopsis and tobacco plants expressing the double T14A/Y15F mutant of CDC2aAt that is thought to be constitutively active. These plants, unlike those expressing *cdc25*, develop normally, except for some tendency toward a reduced apical dominance, but unfortunately they have not been analyzed cytologically. These results suggest either that CDC2aAt is not a substrate of the cytokinin-mediated control of Tyr phosphorylation or, more likely, that there are additional targets, presumably partially redundant with CDC2aAt. The much sought-after enzymes responsible for the phosphometabolism of T14/Y15 in plant CDKs are still awaiting discovery.

The majority of animal CDKs need to be phosphorylated by the so-called CDK-activating kinases (CAKs) for full activation (Harper and Elledge, 1998). Although it is not known whether this type of phosphorylation event is necessary to activate plant CDKs, the kinase CAK1At from Arabidopsis, which is only distantly related to the animal CAKs, has been demonstrated to possess CAK activity toward human CDK2/cyclinA complexes and to complement CAK-deficient mutants in both budding and fission yeast (Umeda et al. 1998). Similar to the budding yeast CAK1 kinase, CAK1At is not cyclin dependent.

There is an indication that the mechanisms of CDK activation may differ between monocotyledonous and dicotyledonous species. Yamaguchi et al. (1998) have found that the rice CDK R2, 50% identical to the animal CAK kinase CDK7, complements CAK deficiency in budding (but not fission) yeast and phosphorylates in vitro the rice CDC2Os1 and the human CDK2 with specificity identical to that of the human CAK. This observation is in conflict with a previous report that R2 has no CAK activity and instead phosphorylates efficiently the C-terminal domain of the large subunit of RNA polymerase II (Umeda et al., 1998).

Proteolytic Degradation

In the course of the past few years, controlled proteolysis has come into prominence as one of the most essential mechanisms underlying cell cycle transitions in eukaryotes (Peters, 1998). Very little is known about this aspect of cell cycle control in plants, primarily because the protein levels of plant cell cycle regulators have not been adequately characterized. From what is currently known, we can only surmise that certain B-type CDKs, for example, CDC2bAt, may be subject to proteolytic removal in the S and early G2 phases, given the considerable delay in the accumulation of the protein compared with the transcript (Figure 1).

Nevertheless, there is little doubt that this form of control exists in plants because (1) the ubiquitin-dependent proteolysis system is present in plant cells, and the expression of some of its elements has been linked to cell proliferation (Plesse et al., 1998); (2) plant A- and B-type cyclins feature the so-called destruction box, a hallmark of ubiquitin-mediated degradation, which has now been found sufficient for cell cycle–dependent protein instability in tobacco suspension–cultured cells (Genschik et al., 1998); and (3) PEST sequences, rich in proline (P), glutamate (E), serine (S), and threonine (T), are also portents of protein degradation and are found in both plant CDKs and cyclins.

In animals and yeast, two multisubunit E3 ubiquitin ligases, SCF and APC, have been found essential for the degradation of a number of cell cycle proteins, including cyclins and CKIs (Peters, 1998). Although homologs of a number of eukaryotic proteins that are related in function to SCF and APC have been described in plants (Leyser et al., 1993; Ingram et al., 1997; Luo et al., 1997; del Pozo et al., 1998; Porat et al., 1998; Ruegger et al., 1998; Xie et al., 1998), their destructive function and role in the cell cycle remain entirely speculative. Intriguingly, two of the plant homologs, AXR1 and TIR1, are implicated in auxin responses (Leyser et al., 1993; Ruegger et al., 1998). This observation raises the attractive possibility that auxin promotes cell division by triggering degradation of CKIs. However, a word of caution is necessary here because homologs of, for instance, TIR1 are involved in a diverse range of functions not necessarily related to the cell cycle.

Although the evidence for protein degradation as a universal mechanism in cell cycle control is accumulating, differences in the mechanisms of degradation of cell cycle proteins in plants compared with other eukaryotes are also anticipated. For example, some maize cyclins that bear a destruction box have been found to be resistant to proteolysis in anaphase (Mews et al., 1997). This finding implies the existence of an active mechanism selectively protecting plant cyclins against proteolysis in M phase.

FUNCTIONS?

The question mark in the title of this section is indeed necessary. Whereas a considerable amount of data implicates plant cyclins and CDKs in cell division control (as discussed above), the links between particular proteins and specific events during the cell cycle remain elusive. The subcellular localization of CDKs and cyclins provides some hints as to their potential functions (Table 3). Complexes of A-type CDKs and B1 cyclins of the CYCB1;2 subtype, for example, are very probably responsible for PPB disintegration, given that they both associate transiently with the PPB immediately beforehand (Hush et al., 1996). These same cyclins, but not the A-type CDKs, colocalize with the condensing chromosomes and the nuclear envelope before its breakdown and thus may be involved in the two processes.

By contrast, cyclin A1, in complexes with a succession of various CDK partners, may well control microtubule dynamics, as suggested by its association with all appropriate structures throughout the cell cycle. On the basis of their spatial (A-type) or temporal (B-type) expression patterns, neither A- nor B-type CDKs qualify as potential partners of cyclin A1 during the early interphase. This conclusion further invokes the presence of additional types of CDKs in the control of the plant cell cycle. Finally, A-type CDKs in complexes with cyclins B1 (CYCB1;1 subtype) and B2 are expected to phosphorylate nuclear proteins.

Growing evidence suggests that pRB-like proteins in plants might be among nuclear targets of plant CDKs. The pRB is central to the regulation of the G1-to-S transition in mammals. Phosphorylation of pRB by cyclin D- and cyclin E-dependent kinases renders it inactive as a repressor of the S phase and thereby promotes DNA replication (Mittnacht, 1998). Significantly, the pRB binding motif LXCXE (where X denotes any amino acid) is found in all known plant D cyclins. Moreover, LXCXE-dependent interactions between D cyclins from Arabidopsis and maize pRB proteins have been demonstrated in vitro and in a yeast two-hybrid assay (Ach et al., 1997; Huntley et al., 1998). The maize pRB proteins contain multiple putative CDK phosphorylation sites, and ZmRB-1 is efficiently phosphorylated in vitro by mammalian G1- and S-specific CDKs (Huntley et al., 1998). Moreover, maize pRB proteins are known to undergo changes in phosphorylation during the transition to endoreduplication in the endosperm (Grafi et al., 1996); however, phosphorylation by plant CDKs remains to be demonstrated.

Further evidence in support of a functional role for pRB proteins in plants comes from experiments showing that the overproduction of a maize pRB-like protein inhibits geminivirus replication. This suggests that pRB-like proteins in plants may also act as negative regulators of DNA synthesis (Xie et al., 1996). The observation that a pRB protein in maize leaves is highly produced in differentiating but not in proliferating cells (Huntley et al., 1998) suggests that some plant pRB-like proteins may be involved in the suppression of cell division during differentiation.

In mammals, hyperphosphorylated pRB disengages from inhibitory complexes with proteins such as E2F and MCM7 that are involved in the activation of S phase–specific transcription (Helin, 1998) and initiation of DNA replication (Leatherwood, 1998). In this regard, the *PROLIFERA* gene, which is required for megagametophyte and embryo development in Arabidopsis, encodes a protein that is 50% identical to the mammalian MCM7 proteins (Springer et al., 1995); it will thus be important to see whether PROLIFERA interacts with pRB proteins in plants.

Similarly, a putative homolog of mammalian E2Fs has recently been identified in wheat (Gutierrez, 1998), and there is an indication that, as is the case in animals, an E2F-like protein might also be a substrate for S phase–specific CDKs in maize endosperm (Grafi and Larkins, 1995). These observations, again circumstantial, imply that the control of the S phase in plants is more similar to that occurring in animal cells than in yeast cells.

PERSPECTIVES

Our understanding of the basic mechanisms that regulate cell division in plants has advanced considerably in recent years. Numerous key players have been identified, and an emerging model that integrates current knowledge is shown in Figure 2. Although initial investigations of the plant cell cycle appeared to be merely confirmatory, the field is now approaching a degree of maturity such that questions specific to plants may be addressed. Given the considerable differences between plants and animals in life strategies, we can expect numerous exciting breakthroughs in the near future. Further progress will depend on gaining a better understanding of the specific roles of the CDK/cyclin complexes. We need to find out which CDK/cyclin combinations are active over the course of the cell cycle and what their targets are. Furthermore, insights into the mechanisms of activation/deactivation should be gained and upstream regulators identified.

These formidable tasks will require considerable efforts in biochemistry and cell biology, efforts that will certainly pay off in the long run. Indeed, a thorough understanding of the operation of the basic cell cycle machinery promises to provide the information and tools necessary to understand how intrinsic developmental programs and environmental cues impinge on cell division. Early payoffs are already emerging as links have been found between auxin signaling and genes known to be involved in cell cycle–related protein degradation (Leyser et al., 1993; del Pozo et al., 1998; Ruegger et al., 1998) and between the action of cytokinins and the regulation of CDK activity (Zhang et al., 1996). We can look forward to understanding how cell division is initiated, how endoreduplication is regulated, and how cells exit the cell cycle to differentiate.

The cell cycle toolbox will also allow us to address fundamental questions with regard to the role of cell division in plant growth and architecture. For example, is cell division informed by growth, or is cell division the driving force for growth? This hotly debated subject pursued for quite some time by plant biologists has fueled arguments denying any role for the control of cell cycle in plant development and reducing cell division to the surveillance of cell growth (Clark and Schiefelbein, 1997).

Current research continues to fuel this debate. For example, in stressed cells of intercalary meristems of rice and of wheat leaves, modulations of the cell cycle have recently been shown to precede any detectable changes in cell growth (Lorbiecke and Sauter, 1998; Schuppler et al., 1998). These findings provide additional evidence that cell growth in plants is not the only driving force for cell division. Further progress in cell cycle research holds the promise of not only bringing a deeper understanding of how, when, and why plant cells divide but also of how cell division in plants might be modulated.

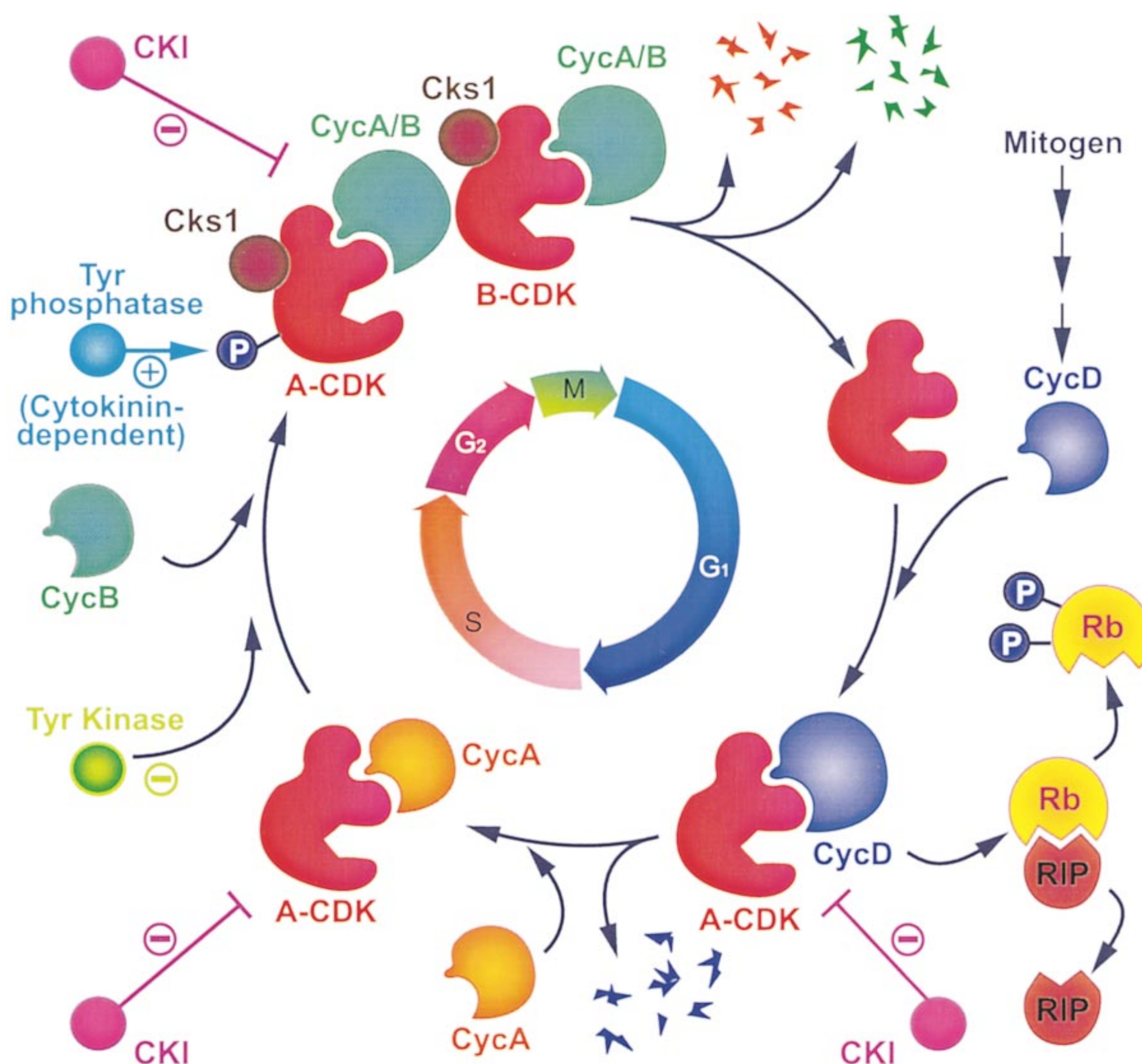

Figure 2. Model of Cell Cycle Control in Plants.

In consideration of the available data on plant cell cycle proteins and genes and in light of data on the corresponding gene products from heterologous systems, a model for cell cycle regulation in plants is proposed. Upon mitogenic stimulation (top right), D-type cyclins (CycD) are produced and associate with the A-type CDKs (A-CDKs). The resulting A-type CDK/cyclin D complexes phosphorylate a retinoblastoma-like protein (pRb), resulting in the release of pRB-interacting proteins (RIP) that in turn trigger the onset of S phase. The presence of PEST degradation sequences accounts for the short life span of the D-type cyclins. During the S phase, A-type cyclins (CycA) are synthesized to activate A-CDKs. As cells reach the end of the S phase, CDK activity is inhibited by Tyr phosphorylation. At G2, B-type cyclins appear. Because both A-type CDKs and B-type CDKs (B-CDK) display kinase activity at the G2/M transition point, both types of CDKs might coincidentally be present to associate with B-type cyclins. The correct functioning and activation of the mitotic CDKs require association with CKS1 docking factors (Cks1) and removal of the inhibitory phosphate group. The latter process was demonstrated to be cytokinin dependent. Specific degradation motifs in their protein sequences suggest that A- and B-type cyclins are destroyed during M phase, as indicated by colored fragments. The cell cycle could be arrested by the association of the A-type CDKs with CKIs.

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