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THE PLANT CELL CYCLE

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■ **Abstract** Cell division in plants is controlled by the activity of cyclin-dependent kinase (CDK) complexes. Although this basic mechanism is conserved with all other eukaryotes, plants show novel features of cell-cycle control in the molecules involved and their regulation, including novel CDKs showing strong transcriptional regulation in mitosis. Plant development is characterized by indeterminate growth and reiteration of organogenesis and is therefore intimately associated with cell division. This may explain why plants have a large number of cell-cycle regulators that appear to have overlapping and distinct functions. Here we review the recent considerable progress in understanding how core cell-cycle regulators are involved in integrating and coordinating cell division at the molecular level.

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INTRODUCTION

Although it seems obvious today, the concept that the cells of an organism increase by the division of existing cells is relatively new because only in 1850 was it shown that cells originate by the cleavage of preexisting cells, and Virchow's aphorism "*omnis cellula e cellula*" (every cell from a preexisting cell) became the foundation of today's theory of tissue formation (78).

During the past two decades, substantial progress has been made in our understanding of the molecular mechanisms of cell proliferation, revealing that conserved or similar fundamental mechanisms are operational at the core of the cell-division cycle of all eukaryotes. However, current studies of developmental processes, coupled with the availability of whole genome sequences, have led to the view that the mechanisms of pattern formation have evolved independently in plants and animals (86). It is therefore to be expected that cell-cycle controls differ between plants and other groups, not only in their details but also in the mechanisms by which developmental and environmental influences on cell division interact with cell-cycle control. These plant-specific aspects of cell division are now starting to be unravelled, and in this review we survey our current knowledge of the molecular processes underlying cell division in plants and briefly address potential control mechanisms of the cell cycle by plant hormones.

LANDMARKS OF THE PLANT CELL CYCLE

The mitotic cell cycle encompasses four sequential ordered phases that temporally distinguish the replication of genetic material from the segregation of duplicated chromosomes into two daughter cells. Lag or gap (G) phases therefore separate the replication of the DNA (S phase) and the segregation of the chromosomes (M phase, mitosis). The G_1 phase (the first gap) intercedes between the previous mitosis and the entry into the next S phase, whereas the G_2 phase separates the S phase from the subsequent M phase. Cells in G_2 are therefore discriminated from G_1 cells by possessing a double DNA content. The gap phases allow the operation of controls that ensure that the previous phase has been accurately and fully completed, and not surprisingly the major regulatory points in the cell cycle operate at the G_1/S and G_2/M boundaries, which correspond to points of potential arrest as a consequence of evaluation of external conditions (135a).

Cell division requires an array of complicated processes that must be executed in a spatially and temporally controlled manner. During G_1, cells must integrate relevant signals before making the decision to initiate DNA duplication, which implies commitment not only to S phase but also to completion of cell division. DNA replication is initiated following the G_1/S transition, and after the doubling of the genetic material, the mechanical structures allowing separation of the chromosomes have to be put in place, starting with the reorganization of the cytoskeleton early in G_2 (140).

At the onset of mitosis, the interphase arrays of cortical microtubules that are arranged tranversely with respect to the main axis of growth rearrange into a narrow cortical ring, the preprophase band. This is unique to plants and consists of a belt-like arrangement of microtubules and actin filaments encircling the future division plane. The preprophase band dissolves as the mitotic spindle is built, which segregates the chromosomes during anaphase. The microtubules then rearrange again to form the phragmoplast, which organizes the synthesis of the new cell wall required between the daughter cells. Phragmoplast assembly starts centrally and expands with the growing cell wall toward the exterior of the cell (66).

Differentiating plant cells often display an alternative cycle known as endoreduplication, characterized by an increase in the nuclear ploidy level that results from repeated S phases with no intervening mitosis (61). In some species, such as *Arabidopsis*, this produces ploidy levels up to 32C in the final stages of leaf development (39). It is also a characteristic of certain specialized cell types, such as trichomes and the cells within leguminous nodules that host nitrogen-fixing bacteroids (37). In all cases, endoreduplication appears to occur only after cells have ceased normal mitotic cycles (24, 37) and in general may be associated with the benefit from increased DNA content to support larger cytoplasmic volumes.

SYNCHRONOUS CELL SYSTEMS

Within the shoot apices of intact plants cell division is essentially asynchronous and there is little or no coordination of division timing between cells (35). Although certain cellular aspects, including the intracellular localization of proteins, can be studied in individual cells and therefore do not require consistent timing of division between cells, the biochemical and molecular analysis of cell division is predicated on the availability of cell systems that allow populations of cells to be synchronized. Although a certain degree of synchrony can be achieved in intact seedlings or plant tissues by the use of inhibitor/block release [e.g., the work of Sala et al. (111)], the most suitable systems for detailed analysis are in vitro suspension cultures of plant cells, which can be grown in shake flasks (42). In such systems, cell division is removed from any developmental context. Ideally, all cells progress through the cell cycle at the same rate from the same initial starting point, and various procedures have been developed for different cultures that allow an approximation of this ideal. These procedures involve some method of accumulating cells at a specific point in the cycle, followed by the reactivation of cell-cycle progression.

The major approaches are the use of reversible inhibitors of specific cell-cycle processes to block progression through the cycle, followed by release of the synchronized cells, and the removal and resupply of specific nutrients or other factors that may be combined with cycles of subculturing. The application of inhibitors to cell-cycle studies has been reviewed recently (96); the most important agents are the DNA-polymerase inhibitor aphidicolin, which blocks cell-cycle progression in early S phase, and the antimicrotubule herbicides such as propyzamide, which block cells in early mitosis (89).

A number of cultures capable of various degrees of synchronization have been developed in species including *Acer* (65), *Catharanthus roseus* (3), alfalfa (64a), *Nicotiana plumbaginifolia* (146), and soybean (75). However, the gold standard of synchronizable cultures, providing not only the best levels of synchronization in plants but also in all higher eukaryotes, is the tobacco Bright Yellow-2 (BY-2) cell line developed by Nagata and coworkers (89), which has become a major factor in the progress of understanding plant cell division. This culture is readily synchronizable with aphidicolin to yield cells that retain a degree of synchrony for more than one complete cycle, and mitotic synchrony can be further enhanced by adding an additional block/release step with propyzamide.

Cultures of the key plant species *Arabidopsis* are recalcitrant to the development of effective synchrony, but we have recently selected cultures that offer sufficient levels of synchrony for molecular and biochemical analysis, using both aphidicolin and nutrient-induced synchrony (80, 81).

GENERAL PRINCIPLES OF CELL-CYCLE CONTROL

The eukaryotic cell cycle is regulated at multiple points, but all or most of these involve the activation of a special class of serine-threonine protein kinases, which are functionally defined as requiring binding for activity to a regulatory protein known as a cyclin and are therefore named cyclin-dependent kinases (CDKs). Yeasts have a single CDK responsible for cell-cycle control that possesses the canonical sequence PSTAIRE (single amino-acid code) within its cyclin-binding domain. This CDK is conserved in all eukaryotes and is hence often referred to by its fission yeast name of *cdc2*. However, in higher eukaryotes there are multiple additional CDKs that have roles at different points in the cell cycle; these CDKs are not conserved between animals and plants and have variant sequences in their cyclin-binding domain. In animals the nomenclature CDK1 to CDK7 has been adopted (95), whereas in plants the lack of direct equivalents (except between CDK1 and CDKA) has led to the adoption of an alphabetical suffix [CDKA to CDKE (62)], which replaces an earlier confused nomenclature based on *cdc2*.

The first cyclins were identified in sea urchin eggs as proteins whose levels fluctuate during the cell cycle and were so named because of this transitory and cyclical appearance (33). They provide the primary mechanism for control of CDK activity because the CDK subunit is inactive unless bound to an appropriate cyclin (Figure 1). Cyclins are a diverse group of proteins with low overall homology that share a large, rather poorly conserved region responsible for their interaction with the CDK; this region is referred to as the cyclin core. The cyclin core stretches about 250 amino acid residues and is organized in two folds of five helices. The first fold is the cyclin box and comprises ~100 amino acid residues (93), representing the region of highest conservation, although it contains only five absolutely invariant positions. The crystal structure reveals the cyclin box as the face of interaction with the cognate CDK (60).

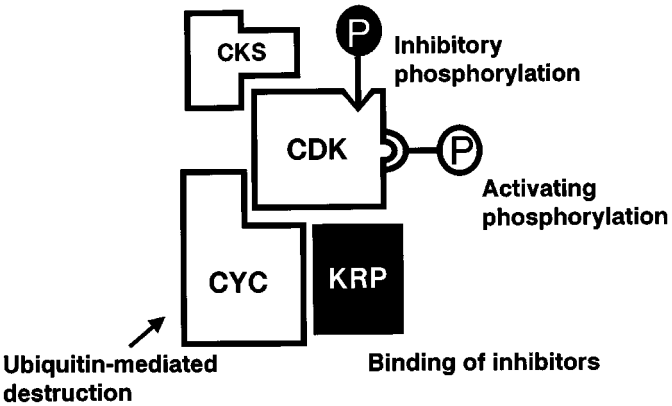

Figure 1 Cyclin-dependent kinase (CDK) activity is regulated at multiple levels. Monomeric CDK lacks activity until it is complexed with cyclins (CYC) and activated by phosphorylation by CDK-activating kinase (CAK). Its activity can therefore be regulated by the abundance of the CDK or cyclin components through controlled transcription, translation, intracellular localization, or regulated destruction, or by the regulated activity of CAK. In addition, activity can be inhibited by phosphorylation by WEE1 kinases or the binding of inhibitor proteins [Kip-related proteins (KRP)]. Inhibitors may block the assembly of CDK/cyclin complexes or inhibit the kinase activity of assembled dimers. CDK subunit (CKS) proteins scaffold interactions with target substrates.

Cyclins fall into many classes that share homology and, at least to some degree, conserved function between animals and plants. A classification based on sequence organization indicates that five types of cyclins exist in plants (A, B, C, D, and H types) (105, 134). A-type cyclins generally appear at the beginning of S phase, are involved in S-phase progression, and are destroyed around the G_2/M transition. B-type cyclins appear during G_2, control G_2/M and mitotic transitions, and are destroyed as cells enter anaphase. D-type cyclins control progression through G_1 and into S phase and differ from A and B types by generally not displaying a cyclical expression or abundance; their presence appears to depend on extracellular signals that stimulate or maintain division. If such signals are removed, levels of D-type cyclins decline rapidly, resulting in cells remaining blocked in G_1. In animals E-type cyclins are strongly regulated at the G_1/S boundary, but a direct equivalent has not been observed to date in plant cells.

The levels of cyclins are generally determined by highly regulated transcription as well as by specific protein-turnover mechanisms. A- and B-type cyclins possess a “destruction box” that targets their timely removal by the anaphase-promoting complex during early-to-mid mitosis. D-type cyclins are conjugated to ubiquitin by an SCF complex and then subjected to proteasome degradation.

No crystal structure of a plant CDK has been reported, but all eukaryotic CDKs share substantial structural similarity, characterized by a bilobal structure. The catalytic cleft, with ATP- and substrate-binding sites, lies between the N-terminal and C-terminal lobes, and cyclin binding stabilizes the catalytic site within the otherwise rather flexible structure [reviewed by Joubès et al. (62)]. However, access to the active site remains restricted by a loop of the CDK known as the "T-loop." Phosphorylation of a conserved threonine residue within the T-loop induces a conformational change that allows proper binding of the substrate and access to the γ-phosphate group of bound ATP. Phosphorylation of Thr160, or the functionally equivalent residue, is therefore essential for CDK activity and is carried out by a CDK-activating kinase (CAK). Two forms of CAK are known, one form classified as CDKF (131, 134), that can substitute for the CAK mutation in yeast, as well as heterodimeric complexes consisting of CDK7 and cyclin H (animals) or their homologues CDKD/CYCH in *Arabidopsis*.

Further regulation of CDK activity occurs by inhibitory phosphorylation of amino-terminal residues around threonine-14 and tyrosine-15, which is catalyzed by the WEE1 kinase, recently reported in maize and *Arabidopsis* (122, 127, 134). In yeast and mammals activation of CDK activity occurs through a phosphatase known as CDC25. Although homology searches do not reveal a direct homologue in plants, it is likely that a functional equivalent is present.

Thus, the activity of the basic CDK-cyclin module is potentially controlled not only through the levels of cyclins and CDK, owing to regulated transcription, translation, protein turnover, and/or sequestration and intracellular localization, but also by activating (CAK-mediated) and inhibitory (WEE1-mediated) phosphorylation. However, further mechanisms of modulation exist through the action of inhibitor and scaffolding proteins. CDK inhibitors have key roles in controlling cell-cycle progression, and there is limited sequence conservation between the animal class of CKI, known as Kip, and plant CDK inhibitors. Plant proteins are known as ICK (inhibitors of CDK) or KRP (Kip-related proteins) and appear to bind both CDK and cyclin subunits (136). CDK subunit (CKS) proteins related to budding yeast $p16^{suc1}$ also exist in plants and animals. These proteins can act as inhibitors and activators of CDK activity and appear to have a role in scaffolding the interaction of the CDK complex with substrates (7).

CDK activity results in the covalent addition of a phosphate group to a substrate protein at a serine or threonine residue, which modifies the substrate's properties. A well-known example of a substrate that is phosphorylated at a specific cell-cycle phase is the extensive phosphorylation of histone H1 in mitotic chromosomes; histones are therefore a commonly used substrate for CDK/cyclin phosphorylation assays. Other known CDK targets include components of the cytoskeleton and the retinoblastoma (Rb) protein, whose phosphorylation controls the G_1/S transition. In other cases, CDK phosphorylation results in the destruction of specific cell-cycle components because recognition of targets for ubiquitin-mediated proteolysis by F-box proteins normally requires previous phosphorylation. In this way, the cell cycle can be given direction because the activation of CDK

activity results in the destruction of components needed for the previous phase (65a).

CORE REGULATORS OF THE PLANT CELL CYCLE

Cyclin-Dependent Kinases

The sequencing of the complete *Arabidopsis* genome has allowed the full complement of a plant's cell cycle genes to be defined. In *Arabidopsis* there appear to be four types of CDK and two types of CAK (62, 134).

The functions of CDKA and CDKB are the best documented. In *Arabidopsis* there is a single gene for CDKA; it carries the PSTAIRE amino acid hallmark, and as in other plants, the abundance of CDKA mRNA does not fluctuate significantly throughout the cell cycle (36, 81, 114, 123). CDKA kinase activity is upregulated during G_1 phase and is high during S phase. Results in tobacco BY-2 cells suggest that activity remains high throughout G_2 until the G_2/M transition (104, 123), whereas in *Arabidopsis* cells there appears to be a reduction in activity during G_2 and a second peak at the G_2/M transition (81).

The expression of A-type CDK in plants is confined to tissues in which the cells have retained the capacity to divide (36, 114). However, CDKA activity within cells is not regulated at the level of its expression because constitutive overexpression does not provoke a phenotype (49). Rather its activity is regulated posttranslationally, and constitutive overexpression of a dominant-negative mutant form of *Arabidopsis* CDKA protein, which does not possess kinase activity, causes reduced cell number in transgenic tobacco plants (49). These and other results suggest that CDKA activity is rate limiting and essential for plant cell division, and although some of its cyclin partners are known, it is not clear with which of these CDKA performs its essential role(s).

Immunolocalization experiments, and more recently the use of a CDKA-green fluorescent protein (GFP) fusion in tracking and cosedimentation experiments, provided detailed information on the intercellular localization of CDKA during the cell cycle (15, 125, 141). During interphase CDKA-GFP is detected in the nucleus and the cytoplasm with the nuclear CDKA-GFP tightly associated with chromatin. During late G_2 CDKA-GFP is briefly associated with the cortical portion of the preprophase band. During prophase it is detected in the condensing chromatin, and during prometaphase it is recruited to the metaphase spindle, a result also confirmed in cosedimentation experiments. At anaphase CDKA is localized to the central region of the spindle; during telophase the CDKA accumulates on the midline of the phragmoplast. At later stages of telophase CDKA reassociates with the chromatin (141). This close involvement of CDKA with the rearrangements of the microtubules that accompany mitosis suggests it has a key role in controlling microtubule dynamics and hence also mitotic progression (126).

B-type CDKs are unique to plants, and the *Arabidopsis* CDKBs are subdivided in two subgroups, each with two members (134). The expression of both CDKB1

and CDKB2 has been demonstrated and documented (80, 81, 98, 114, 123). Both subgroups exhibit cell-cycle regulation of their expression, a feature that is unique to plant CDKBs and has not been observed for any type of CDKs in other eukaryotes.

The CDKB1 subgroup normally has the hallmark sequence PPTALRE and is expressed from the onset of S phase until mitosis in a number of species examined including *Antirrhinum* (*cdc2c*) (36), alfalfa (*cdc2MsD*) (76), tobacco (123), and *Arabidopsis* (81, 114). The CDKB2 type is normally characterized by PPTTLRE and is expressed only in G_2-M cells where examined in *Antirrhinum* (*cdc2d*) (36), *Arabidopsis* (81), and alfalfa (*cdc2MsF*) (76).

It is presumed that CDKB1 and CDKB2 kinase activity is limiting for the G_2/M transition. Constitutive overexpression of a dominant-negative mutant of a CDKB in tobacco increased the proportion of cells with a 4C content, indicating that cell cycle progression is inhibited some time prior to nuclear membrane degradation (98). By in situ hybridization, CDKB expression is confined to actively dividing tissues and is dispersed in a patchy pattern in these tissues, indicative of a cell-phase-specific accumulation. Its expression is excluded from endoreduplicating tissues (58, 63). Furthermore, double-labeling experiments in *Antirrhinum* revealed the accumulation of *CDKB1* transcripts in cells in S, G_2, and M phase and of CDKB2 transcripts in cells in G_2 and M (36). Activity peaks at the G_2/M transition (123). Immunolocalization of alfalfa CDKB2;1 (*cdc2MsF*) revealed colocalization with microtubular structures such as the preprophase band, preprophase spindle, metaphase spindle, and phragmoplast (83). CDKs may also function outside the cell cycle, as downregulation of *Arabidopsis* CDKB1;1 reduced the growth of etiolated seedlings primarily by inhibiting the elongation rates, independently of cell division or endoreduplication (145).

C-type CDKs are most closely related to the cholinesterase-related cell-division controller (CHED) kinases of mammalian cells; they share the PITAIRE motif with these proteins, and no function in the cell cycle has yet been demonstrated (73a). In mammals, CHED kinases are suspected to be involved with megakaryocyte differentiation in hematopoiesis (71). Tomato CDKC failed to interact with A, B, or D cyclins, and although it is preferentially expressed in dividing tissues, its expression pattern does not indicate a cell-phase-specific regulation (63). In alfalfa the expression of CDKC is also constant throughout the cell cycle (76). However, no loss-of-function data are yet available to definitely rule out a cell-cycle function of this type of CDK.

The SPTAIRE CDK, found in alfalfa, was classified as an E-type CDK (62), and on the basis of homology a related sequence was detected in *Arabidopsis* (134). Their possible function in the cell cycle is still unclear. CDKE maintains an unchanged basal level throughout the cell cycle (76).

CDK-Activating Kinases

The first plant CAK, R2, was identified in rice (45, 112, 144) and served as a model to identify three related sequences in the *Arabidopsis* genome that were classified

into the group of D-type CDKs (134). Additionally, a further, unrelated *Arabidopsis* CAK was isolated in a yeast complementation assay, and the kinase activity of immunoprecipitates with anti-CAK antibody toward CDKA was confirmed. Unlike monomeric yeast CAK, the *Arabidopsis* CAK did not display activity as a monomer, suggesting the need for a posttranslational modification or the association with a regulatory subunit for activity (131). Because the sequence of this *cak1At* was unrelated to CDKD and the rice CAK R2, apart from its conserved kinase domain, it was recently classified in a distinct group, CDKF (134). It therefore appears that there are two classes of CAK in *Arabidopsis*. Rice CAK R2 not only has kinase activity toward CDK but also phosphorylates the C-terminal domain of RNA polymerase II. R2 is preferentially expressed during S phase, and the kinase activity associated with R2 using the carboxy-terminal domain as a substrate peaks during S phase (34). Unlike rice R2, the *Arabidopsis* CDKF;1 complexes cannot phosphorylate the carboxy-terminal domain, but specifically phosphorylate CDKs (131). Inducible cosuppression of *Arabidopsis* CDKF using either sense or antisense constructs led to a decrease of CDK activity and caused a premature differentiation in root cells (132). The use of the specific CDK inhibitor roscovitine also caused differentiation, but other blockers of the cell cycle such as aphidicolin did not. This suggests that continued CDK activity (rather than cell division itself) is directly important in maintaining the undifferentiated state of meristematic cells.

Cyclins

A-TYPE CYCLINS A-type and B-type cyclins could be isolated in a variety of plant species by polymerase chain reaction (PCR)-based cloning strategies, benefiting from the relatively conserved cyclin box (46, 48). Although early analyses (based on a limited numbers of sequences) suggested ambiguities in the assignation of cyclins between the A and B subgroups, later, more detailed studies showed that animal and plant A- and B-type cyclins show clear relationships in their overall sequence and the nature of their destruction box (105, 106). Levels of A-type cyclins, like B-type cyclins, are tightly controlled by cell-cycle-dependent proteolysis, and this is conferred by the N-terminal destruction box (41).

A-type cyclins are subdivided into three different subclasses: CYCA1, CYCA2, and CYCA3 (12, 105). In *Arabidopsis* 10 sequences encoding A-type cyclins have been identified; four of these are predicted by bioinformatics, and the expression of the other six has been confirmed (134). In most plants screened, members of all three subclasses of A-type cyclins have been found.

Expression analysis in various models indicated that A-type cyclins are expressed before B-type cyclins in the plant cell cycle, beginning around the onset of S phase (38, 56, 70, 82, 103, 116). In tobacco BY2 cells, sequential expression of different A-type cyclins was analyzed during the cell cycle. The expression of two A3-type cyclins was upregulated at the G_1/S transition, whereas a gene belonging to the A1-type was induced at mid-S phase. These data suggested that the members of the different subclasses have different biological functions (103).

An alfalfa *CYCA3* (*CycMs3*) was expressed prior to DNA synthesis upon cell-cycle reentry of differentiated cells and could complement a G_1-cyclin-deficient yeast, suggesting a possible role in cell-cycle reentry or the G_1/S transition (82). Although expression timing is consistent with this interpretation, the ability to rescue G_1-cyclin-deficient yeast cells should not be regarded as a criterion for cyclins involved in G/S control because B-type cyclins, which are not expressed during G_1, can share this potential (20).

Localized induction of *CYCA3;2* expression stimulated cell division in primordia and young organs in tobacco, suggesting that in certain situations CYCA activity can be rate limiting for cell division. Intriguingly, this transient local induction in cell division in primordia led to a reduction in cell number in the cognate region of the mature leaf (142), suggesting that control of cell division in localized domains is important for proper morphogenesis.

A detailed study of the expression kinetics of A2-type cyclins in partially synchronized cell suspension cultures revealed that transcript levels peaked during G_2, dropped during M phase, and then rose again during G_1 and S. CYCA2 protein levels dropped during G_1, rose during S, and then remained at a constant level during G_2 and M phase. However, the CYCA2-associated kinase activity was biphasic; it transiently peaked during mid-S phase, then dropped to a background level and reached its maximum level at the G_2/M transition (110).

Intriguingly, CYCA2 was shown to be able to interact with the maize retinoblastoma protein in a yeast two-hybrid experiment. This interaction requires an intact cyclin-box, indicating that a yeast CDK probably forms a complex with CYCA2, and this heterodimer interacts with the Rb instead of directly with the CYCA2 (110).

A detailed expression analysis of *Arabidopsis CYCA2;1* expression in the root pericycle indicated that it was associated with the pericycle cells at the xylem poles, which arrest in G_2, and was not detected with the pericycle cells at the phloem poles, which remain in the G_1 phase of the cell cycle (5). Although a role for CYCA-kinase activity in the G_1/S transition certainly cannot be excluded at this point, particularly in the case of *CYCA3*, it seems from the maxima of analyzed CYCA-associated kinase activities during both S and G_2, and their expression pattern in tissues, that they are predominantly involved in S and G_2-phase progression rather then in G_1 progression.

Details on subcellular localization of CYCAs during the cell cycle are starting to emerge, but we are still far from a complete picture. During S phase a CYCA3; 1-GFP fusion protein was restricted to the nucleus and nucleolus; in cells undergoing mitosis no CYCA3;1-GFP was detected (18). Similarly, in alfalfa CYCA2 was detected in the nucleus until prophase but was undetectable during mitosis (110). Immunolocalization experiments in *Zea mays* indicated that CYCA1 becomes nuclear after prophase and binds to microtubular arrays during mitosis (84, 85). Generally, these data are consistent with distinct roles for the various classes of A-type cyclins.

B-TYPE CYCLINS Among the first cell-cycle genes cloned in plants were three B-type cyclins, two from carrot and soybean (46) and one from *Arabidopsis* (48).

Apart from their sequence differences, B-type cyclins distinguish themselves from A-type cyclins by their somewhat later expression pattern during the cell cycle. All identified B-type cyclins were subdivided into two subclasses, CYCB1 and CYCB2 (105), but recently the presence of a B-type cyclin gene in the *Arabidopsis* genome, which encodes for a B-type-cyclin-like protein without the typical B-type destruction box, was predicted and assigned to a third class, CYCB3 (134). There are two genes encoding for both CYCB1 and CYCB2 cyclins in *Arabidopsis*.

Microinjection of tobacco *CYCB1;1* mRNA into *Xenopus* oocytes overcame their natural G_2/M arrest, as do mammalian cyclins (99). Ectopic expression of the *CYCB1;2*, but not of the *CYCB1;1*, cyclin in normally endoreduplicating trichomes forces the G_2/M transition and causes the trichome cell to convert what would normally be endoreduplication cycles into cell divisions producing multicellular trichomes (113), indicating that plant CYCB1 are indeed functionally involved in the G_2/M transition.The mechanism involved in G_2-M-specific transcriptional regulation has been the topic of extensive studies. In synchronized cell suspensions and plants, *CYCB* transcripts are absent until S phase, rise during G_2, reach a maximum during G_2 and early M phase, and decline rapidly as M phase progresses (5, 51, 81, 99, 129). The use of a β-glucuronidase (*GUS*) reporter fused to the full-length *CYCB1;1* promoter indicated that the levels of *GUS* mRNA mimicked the expression kinetics of the *CYCB1;1* in cell systems, showing that the kinetics of B-type cyclin mRNA accumulation are conferred by transcriptional regulation (56, 117, 129). Deletion analysis of the *Catharanthus CYCB1;1* promoter in combination with the luciferase reporter gene allowed the identification of a nine-base-pair element containing a central core pentamer with homology to Myb-binding sites that is responsible for the M-phase-specific regulation. Furthermore, this MSA (M-phase-specific activator) element was conserved in the promoters of several M-phase-regulated genes (57, 130). Three cDNAs encoding Myb proteins (NtMybA1, NtMybA2, and NtMybAB) were isolated from a tobacco BY-2 cDNA library using MSA elements from *Catharanthus* and tobacco as bait in a one-hybrid screen (55). Furthermore, NtMybA1 and A2 activated the MSA-containing promoters, whereas NtMybB inhibited their activity. It therefore appears that Myb factors are conserved MSA-interaction factors and regulate M-phase-specific transcription. However, different factors may regulate other *CYCB* genes, as two interacting proteins, which were found to be a putative Myb-like factor with a Myb-DNA binding domain and a protein that contains a Myc-type dimerization domain and leucine zipper, were identified as binding the *Arabidopsis CYCB1;1* promoter (97).

As mentioned above, levels of B-type cyclins are regulated not only by transcriptional control but also by proteolysis, and CYCB destruction by the anaphase promoting complex marks the transition from metaphase into anaphase in animals, and possibly in plants (41). CYCB protein levels are therefore high only during a narrow window of the cell cycle; tobacco CYCB1;1 protein levels rose at the end of S phase and dropped early in the M phase in a synchronized tobacco BY2-culture (17). Unlike the situation in vertebrate and yeast cells, in which overexpression of a nondegradable form of cyclin B blocks mitosis, in BY-2 cells

nondegradable CYCB1;1 did not profoundly perturb progression through mitosis, nor did it increase CDK kinase activity, but CYCB1;1 negatively affected cytokinesis, resulting in a low frequency of multinucleate cells (18). This indicates that additional mechanisms regulate CDK and CYCB1 activity (17, 41) and that CYCB1 destruction is not a prerequisite for the completion of mitosis.

In G_2 tobacco, CYCB1;1 is either nuclear or cytoplasmic. In prophase, CYCB1;1-GFP fusion protein was found on the condensing chromosomes, and no colocalization with mitotic spindles was observed for this cyclin. As expected, in anaphase and telophase the fusion protein was undetectable because it is destroyed at the metaphase/anaphase boundary (18).

The expression of B-type cyclins in plants is mainly associated with cycling cells (5, 58, 114). Fusion of *GUS* to the promoter and the coding region for the first 50 amino acid residues of *Arabidopsis CYCB1;1*, a region that includes the destruction box, produces a GUS fusion protein that is expressed only in G_2/M cells and is destroyed as cells pass through mitosis (16). This marker is therefore an excellent indicator of mitotic activity in plant tissues. However, the situation at the protein level is more complex, as in maize root tips only the CYCB1;2 protein was completely absent from nondividing tissues; CYCB1;1, CYCB2;1, and CYCA1;1 disappeared from the cytoplasm but persisted in the nuclei of elongating cells at a lower level (85).

Targeted constitutive overexpression of *Arabidopsis CYCB1;1* to division-competent cells promoted cell division in root meristems and increased root growth, indicating that this CYCB1 might be rate limiting for division in these cells (29), whereas ectopic expression of *CYCB1;1* failed to overcome the G_2/M block in endoreduplicating cells (113). However, the mechanism behind these phenomena is unclear and may be linked with the specific ability of some root cells to arrest in G_2 (5).

D-TYPE CYCLINS D-type cyclins were originally identified by their capacity to complement yeast strains defective in G_1 cyclins (19, 120). They were defined as D-type cyclins on the basis of a low sequence homology to animal D-type cyclins and the presence of the conserved LxCxE motif, which is responsible for their interaction with retinoblastoma-related (Rb or RBR) proteins (1, 53). They have been identified in a variety of plant species [see review by Meijer & Murray (79)]; in *Arabidopsis*, ten D-type cyclins sequences have been identified, and these can be classified according to Vandepoele et al. (134) into seven subclasses. Using this classificiation of *Arabidopsis* D-type cyclins, the CYCD3 subclass has three members, the CYCD4 family two, and the other groups all have a single member. The CYCD4 subgroup has relatively high homology with CYCD2;1 (CYCD4;1 and CYCD4;2 have 60% and 54% similarity with CYCD2;1, respectively), similar to the homology shared by other subgroup members (CYCD3;1 shares 61% similarity with CYCD3;2 and 57 % with CYCD3;3), and it has been proposed that the CYCD4 cyclins should be regarded as members of the CYCD2 subgroup (54, 94).

D-type cyclins share conserved structural features with mammalian D-types in addition to the cyclin box, such as the Rb-interaction motif and in most cases putative PEST (proline, glutamine, serine and threonine rich) sequences involved in protein degradation. The Rb-interaction domain consists of an LxCxE (single-letter code, where x represents any residue) amino acid motif that is conserved in both animals and plants and is located near the N terminus of the protein. For CYCD2;1 and CYCD3;1, Rb binding has been demonstrated in vitro and depends on an intact LxCxE motif (1, 53). Two cyclin sequences, CYCD4;2 and CYCD6;1, coding for a CYCD-related protein without a canonical LxCxE motif, have been predicted, and CYCD5;1 has a slightly divergent motif. The biological role of these proteins is still unclear. In *Arabidopsis* both CYCD2;1 and CYCD3;1 form active complexes with CDKA but not with CDKB1;1 (47). Interaction with other possible CDK partners has not been probed yet, although alfalfa CYCD4 associated with the alfalfa CDKB2 in yeast (83).

Expression of D-type cyclins is low in stationary BY-2 cell cultures (121), although in an *Arabidopsis* cell culture *CYCD2;1* and *CYCD3;1* transcripts were constant throughout the growth cycle from subculturing to stationary phase. However, when the culture medium was adjusted so that sugar levels were depleted by the time stationary phase was reached, *CYCD3;1* transcripts dropped significantly. These results show that *CYCD2;1* and *CYCD3;1* expression is not limited to actively dividing cells but rather is dependent on medium composition and hence extrinsic signals. Moreover, together with other evidence, it shows that both *CYCD2;1* and *CYCD3;1* expression is dependent on sugar availability and is induced in response to sugar supply (108). Induction of both *CYCD2;1* and *CYCD3;1* by sugars occurred without progression through G_1 or de novo protein synthesis, as shown by its insensitivity to cycloheximide, a protein synthesis inhibitor that also blocks progression through G_1 phase in *Arabidopsis* (38). This induction is, however, highly sensitive to inhibitors of protein phosphatases used at low concentrations, and the response profiles of CYCD2;1 and CYCD3;1 induction are different to two phosphatase inhibitors, indicative of the involvement of different phosphatases or pathways (108).

Further analysis showed that although *CYCD2;1* and *CYCD3;1* transcript levels drop on sucrose removal, total levels of CYCD2;1 protein remain fairly constant, whereas CYCD3;1 protein levels follow the transcript kinetics (47). CYCD3;1 therefore parallels the classic response of mammalian D-type cyclins to external mitogenic signals (118). However, the kinase activity associated with both CYCD2;1 and CYCD3;1 dropped upon sucrose removal. Immunoprecipitation revealed that in stationary phase CYCD2;1 is not bound to CDKA but is sequestered in an unknown complex that removes CYCD2;1-associated kinase activity without affecting the total protein level (47).

Human D-type cyclins are degraded by ubiquitin-mediated proteolyis that involves phosphorylation of the threonine-286 residue of the protein. Threonine-286 or its equivalent is located within the hydrophilic PEST domain and is conserved in D-type cyclins of various organisms including plants, and therefore a similar

mechanism for the regulation of D-type cyclin levels might be operational in plants (90, 94).

Although both *CYCD2;1* and *CYCD3;1* expression is sugar regulated, *CYCD3;1* expression shows strong responses to plant hormones, whereas *CYCD2;1* does not (94). *CYCD3;1* is rapidly super-induced by cytokinin within one hour of treatment at physiological concentrations, not only in cultured cells (107, 120) but also in whole seedlings, as detected by RNA gel blots and in situ hybridization. *CYCD3;1* expression is also elevated in the high cytokinin mutant *pt/amp1* (13, 107).

However, the hormone response of *CYCD3;1* is not limited to its super-induction by cytokinin, as it is also upregulated by auxin, gibberelins, and brassinosteroids (52, 94). The response to cytokinins, like its sugar response, is phosphatase mediated and does not appear to require de novo protein synthesis (107), in contrast to the induction by epi-brassinolide, which does involve de novo protein biosynthesis but no regulation by phosphorylation (52). This suggests that the brassinolide regulation of *CYCD3;1* requires transcription and/or translation of upstream regulators. Abscisic and jasmonic acids reduce the levels of *CYCD3;1* transcripts in *Arabidopsis* cells, consistent with the effects of these hormones in blocking cell-cycle progression in BY-2 cells (94, 128).

In *Antirrhinum* seedlings, *CYCD1*, *CYCD3;1* (*CYCD3a*), and *CYCD3;2* (*CYCD3b*) respond to sucrose. Interestingly, only *CYCD1* and *CYCD3;1* expression is significantly upregulated by cytokinin. Furthermore, only *CYCD1* displays a mild response to auxin (40).

CYCD3;1 might have an additional role besides integrating sucrose and hormone signaling. Toxin-induced cell-cycle arrest in early S phase by aphidicolin or in G_1 by low levels of cycloheximide causes a strong reduction in *CYCD3;1* transcript levels (38, 81), indicating that *CYCD3;1* expression may also assess correct cell-cycle progression.

In tissues, *CYCD* expression seems to be associated with proliferating tissues and is excluded from differentiated tissues. *CYCD4;1* expression is associated with developing lateral roots, embryogenesis, and vascular tissues (25). *CYCD3;1* transcripts accumulated in proliferating shoot tissues such as the meristematic, young leaf, and developing vascular tissues (107, 28). Furthermore, D-type cyclins of the same class can be differentially expressed in dividing tissues during development. In *Antirrhinum*, *CYCD3;2* (*CYCD3b*) is expressed throughout the vegetative meristem, whereas the cytokinin-inducible *CYCD3;1* (*CYCD3a*) transcripts are confined to early stages in the development of organ primordia. In *Antirrhinum* floral meristems, *CYCD3;1* expression was detected in the periphery of the inflorescence meristem, in young floral meristems, and in the tips of older floral organs. *CYCD3;2* transcripts, however, were abundant throughout the inflorescence meristem and floral meristems, being apparently expressed in all dividing cells. *CYCD1* was also expressed throughout the inflorescence (40).

CYCDs may also provide a key link between developmental controls and cell proliferation. *AINTEGUMENTA* encodes a transcription factor of the AP2 domain family and was identified as a gene that regulates cell division in ovules and flowers (31). Ectopic overexpression of *AINTEGUMENTA* in *Arabidopsis* increases

the size of leaves by extending the period of cell proliferation and increasing leaf cell number and also causes increased expression of *CYCD3;1* (87). Overexpression of *CYCD3;1* also dramatically increased leaf cell number but did not affect the expression of *AINTEGUMENTA* (28), suggesting that *AINTEGUMENTA* may regulate cell number in organ development by controlling *CYCD3* expression.

H-TYPE CYCLINS Genes encoding H-type cyclins have been identified in *Arabidopsis*, rice, and poplar (134, 143). In rice and poplar high transcript levels are associated with dividing cells. Rice cyclin H interacts specifically with the CAK R2 but not with other CDKs, and increases the kinase activities of R2 and enhances the rescue by R2 of a CAK mutation in budding yeast. These observations indicate that cyclin H is the regulatory subunit of CAK (143). Further information on the regulation of CYCH and CAK itself is likely to show whether these proteins have a regulatory role in the plant cell cycle.

CDK Inhibitors

Binding of CDK inhibitors to CDK/cyclin complexes modulates the kinase activity. In *Arabidopsis* the seven genes identified that encode proteins with limited homology to the animal Kip/Cip family of CDK inhibitors are referred to as Kip-related proteins (KRPs) or ICKs (inhibitor of CDK) (24, 74, 137). Apart from the Kip-related region, the overall homology of most KRPs is rather low. The binding of some of these KRPs to CDKA has been reported independently by two research groups, and a consensus result from their experiments was the binding of ICK1, ICK2, and ICK7 to CDKA and the absence of CDKA interaction with ICK5 (24, 148). Also, no KRP was found capable of binding CDKB in a two-hybrid system in these studies. All KRP/ICK proteins interacted with CYCD3;1, CYCD2;1, and CYCD1;1 (148), and four interacted with CYCD4;1 (24). No KRP interacted with CYCB1;1 (24, 148). Inhibition of CDKA activity has been demonstrated in vitro for both ICK1/KRP1 and KRP2 (139). In addition, an in vivo assay demonstrated the ability of ICK1 to delay the progression from nuclear envelope breakdown to metaphase in *Tradescantia* stamen hairs (14). Together these results suggest that some or all KRP proteins act to inhibit formation of CYCD-CDK complexes and to inhibit the kinase activity of preformed CDKA complexes, but there is no direct evidence that KRP binding blocks CDK-cyclin complex formation.

The inhibitory effect of KRP proteins on CDKA activity has been confirmed as also affecting cell-cycle progression in transgenic plants. The targeted overexpression of KRP1 resulted in petals with a reduced cell number (148), and constitutive overexpression of KRP1, KRP2, and KRP4 resulted in smaller serrated leaves (24, 148). Aberrant floral morphology was found upon the overexpression of KRP1 and KRP4 (148). Constitutive overexpression of KRP2 reduced cell numbers in leaves, which was correlated with and hence partially compensated by an increase in cell size. Leaf development is characterized by largely distinct phases in which first cell division and later cellular differentiation and accompanying expansion are

the predominant processes (30). In KRP2-overexpressing plants cell division was inhibited in the first phase, but the timing of the switch between division and cellular differentiation/expansion was unaffected, suggesting no direct role for KRPs in integrating proliferation and cellular differentiation (24).

KRP transcripts are differentially distributed in *Arabidopsis* plants (24, 139), and in synchronized *Arabidopsis* expression systems their transcripts are differentially regulated. During cell-cycle reentry of sucrose-starved cells, *KRP2* is transiently expressed during the G_1 phase, but its transcripts subsequently disappear. *KRP1/ICK1* transcripts are present in sucrose-starved cells, and its transcripts then decline but increase again at the ensuing mitosis, whereas *KRP3* is limited to mitotic cells (81). *KRP1/ICK1* is also strongly upregulated by abscisic acid treatment (138). These results suggest different roles for the KRP proteins during the cell cycle. KRP2 may be specifically involved in cell-cycle reentry, and KRP1/ICK1 in maintaining cells in stationary phase and abscisic acid responses.

CDK Subunit

CDK subunit (CKS) proteins are proposed to act as docking factors that can influence the interactions between the kinase complex and the substrates. Interaction of *Arabidopsis* CKS1 and CDKA and CDKB has been demonstrated (24, 148). The expression of CKS1 (26) is associated with dividing cells as well as with endoreduplicating cells (58). Recently, a second homologue in *Arabidopsis* has been identified by genomic analyis (134).

Retinoblastoma Protein

A single gene for an Rb-related protein exists in *Arabidopsis*, but in maize there are two genes (*RBR1* and *RBR2*) as well as differentially spliced transcripts, although their functional significance is unknown (1). Plant Rb proteins are nuclear and are characterized by many potential CDK phosphorylation sites (1, 22, 91). In animals and plants Rb proteins interact with several proteins including the E2F family of transcription factors, CYCD, and RbAp48-like proteins (1, 22, 53). In maize the *RBR* genes are expressed in all tissues, but the strongest expression is associated with the shoot apex (1). When Rb protein abundance was examined in developing maize leaves, a gradient along the leaf lamina was found, with the highest levels associated with differentiating cells exiting the cell cycle at the distal end of the leaf and lowest levels associated with the proliferative zone at the leaf base (53). The *Arabidopsis* homologue of the Rbp48-related Rb-interacting protein MSI1 has recently been shown to be a component of the CAF chromatin assembly factor complex together with the proteins encoded by the *FAS1* and *FAS2* genes (64b), whose loss leads to fasciation of the shoot apical meristem and inability to maintain the expression of the meristem identity gene *WUSCHEL*. These results suggest links between Rb's role in cell-cycle control and cellular differentiation.

E2F

E2F binds DNA as heterodimers with dimerisation partner (DP) proteins, and homologues of E2F and DP proteins are found in plants and interact with Rb (2, 21, 43, 69, 77, 100, 115). In *Arabidopsis* a family of three E2F protein [E2Fa, E2Fb, and E2Fc, according to the latest nomenclature (134)], two DP (a and b) proteins, and three DP-E2F-like genes [DEL (also known as ELP), with mixed sequence characteristics] have been described (21). E2Fa (also known as E2F3) and E2Fb (E2F1) transactivate gene expression, whereas E2Fc (E2F2) appears to have weak or no transactivation potential (21, 69, 77, 109). E2Fa and E2Fb translocate to the nucleus and transactivate an E2F reporter gene when coexpressed with DPa but not with DPb (69). The DEL proteins do not interact with DP or E2F proteins but can bind to E2F sites in a monomeric form and repress the transcription of genes under E2F promotor control (68). The E2F/DP transcription factors can trigger genes involved in DNA replication. Rice OsE2F1 binds to the putative E2F binding site in the promoter of proliferating cell nuclear antigen, a gene involved in DNA replication, and confers transactivation to an E2F-reporter gene (67); cell-cycle regulation of the tobacco ribonucleotide reductase gene is controlled by E2F elements (10, 11).

Combined overexpression of E2Fa/DPa transcription factors in *Arabidopsis* stimulated the expression of S-phase-specific genes such as DNA polymerase α, ORC, MCM, and CDC6 (23) and stimulated the entry into S phase of fully differentiated leaf cells (109, 23). In leaves this led to either ectopic divisions or increased rounds of endoreduplication; the choice between these two alternatives is proposed to be controlled by the presence or absence of a putative mitotis-promoting factor (23). In trichomes, *CYCB1;2* can provide this role (113), suggesting that this cyclin or a regulator of its expression or activity might provide such an activity.

CELL-CYCLE TRANSITIONS AND PROGRESSION

From G_1 Into S Phase

A model for control of the G_1/S transition has been proposed in which D-type cyclins are primary mediators of the G_1/S transition and hence have a major responsibility for stimulating the mitotic cell cycle (88) (Figure 2). Transcription of D-type cyclins is activated by extracellular signals and leads to the formation of active CDKA-CYCD complexes. These phosphorylate and hence inactivate the Rb protein so that it loses its E2F association and can no longer block the activation of E2F-regulated genes. E2F is then able to activate transcription of genes involved in S phase and other growth and cell-cycle processes (10, 23).

The role of multiple CYCDs during G_1/S control is not yet understood, nor is it known whether Rb is their only important target. Do different CYCD kinases phosphorylate different positions within Rb or do they act sequentially during progression through G_1 into S phase, or in different cell types?

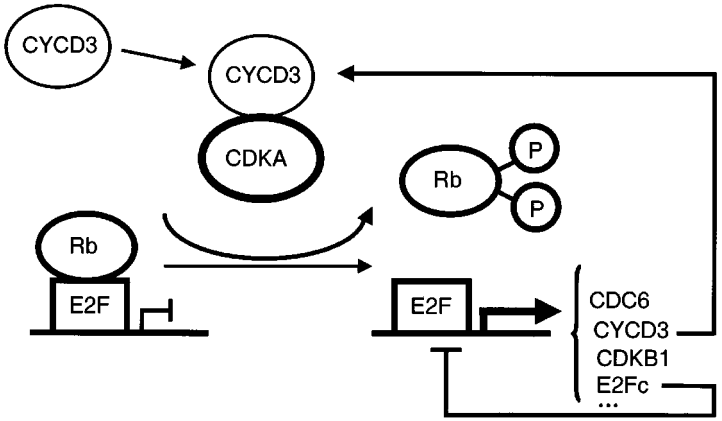

Figure 2 Model for the control of G_1/S transition. CYCD3 transcription is induced in response to sucrose and hormones and forms active complexes with CDKA. In nondividing cells, or cells in G_1, genes with E2F sites are held transcriptionally inactive because bound E2F recruits retinoblastoma (Rb) to these promoters. The rising levels of CYCD kinase activity phosphorylate Rb, causing it to lose E2F association. E2F-regulated genes including *CDC6*, *CYCD3;1*, *E2Fc*, and *CDKB1;1* are thereby activated. The control incorporates positive and negative feedback loops because CYCD3;1 is an E2F target, as is the E2Fc, which lacks transactivation potential and is therefore a competitive inhibitor of E2F-regulated activity.

The role of the multiple E2F genes is also not fully resolved, although it seems likely that E2Fc is involved in a negative-feedback loop. As already mentioned, E2F2c appears to be a competitive inhibitor of E2F-activated gene expression owing to its lack of an effective activation domain, and it contains an E2F-binding site in its promoter. Increasing levels of E2F activation activity would therefore lead to increased competitive inhibition of E2F-directed expression. This model has been supported by the finding that when CYCD3;1 is overexpressed, resulting in Rb inactivation, strongly increased levels of E2Fc are detected (28).

G_2/M Transition and Mitosis

The G_2/M transition is controlled by the level of CDK-CYCB kinase activity, and inhibition of CDKs arrests cells in G_2 and stabilizes the preprophase band (6). During G2 levels of transcripts encoding CYCB increase and probably associate with both CDKA and CDKB1. Further regulation of CDK activity by both KRP proteins and inhibitory phophorylation by WEE1 kinases is likely. Phosphatases therefore probably play an important role in M-phase onset, although a direct homologue of CDC25 phosphatases that activate CDKs in animals and yeast has not been found. However, because inhibition of serine/threonine-specific protein phosphatases in

alfalfa by endothall caused premature, imperfect microtubular reorganizations and activation of CDKB2;1 (4), these phosphatases might be involved in regulating M-phase progression. The protein kinase CK2 shows discrete activity peaks at G_1/S and M in tobacco BY-2 cells, and blocking its activity during G_1 abolished the G_2/M checkpoint, resulting in premature entry into prophase and showing links between G_1 processes and G_2 controls (32).

Progression Through Mitosis

As discussed above, progression through mitosis involves the association of CDK complexes with microtubular and chromatin structures, and CDK activity probably plays an important role in microtubular dynamics and stability (135). Microinjection of active CDK into *Tradescantia* hair cells accelerates the early stages of mitosis, whereas inhibition of CDK activity induces abnormal microtubular spindles (6). Although the actual substrates of CDK have not yet been identified, CDK complexes could phosphorylate microtubule-associated proteins such as MAP4 homologues, which regulate the dynamic assembly of microtubular structures, in analogy with animals.

In animals, cytokinesis occurs by constriction of the cell cortex, but in plants it depends on the phragmoplast, to which cell-plate formation is directed by transport of Golgi-derived vesicles [reviewed by Staehelin & Hepler (124)]. A variety of proteins involved in vesicle trafficking and microtubular-associated proteins accumulate at the phragmoplast. In addition, a number of protein kinases such as MMK3, $p43^{Ntf6}$, and mitogen activated protein kinases (MAPKs) are detected on the cell plate. NPK1, a MAPK kinase kinase that accumulates at the equator of the phragmoplast, regulates the lateral expansion of the cell plate, and its targeted action depends on the association with kinesin-like motor proteins (92).

Endoreduplication

Endoreduplication occurs in the development of mature tissues in many species and in specific developmental situations such as endosperm development (44). Endoreduplication can be considered a modified cell cycle, and data have emerged on the molecular mechanisms behind the regulation of endoreduplication [reviewed by Joubès & Chevalier (61)]. A general view is the downregulation of CYCA1, CYCA2, CYCBs, and CDKB in endoreduplicating cells, which is consistent with the proposed action of these components during M phase. Also, as mentioned above, the overexpression of *CYCB1;2* in endoreduplicating trichomes induces mitotic divisions, indicating that this can be a limiting factor for entry into mitosis.

CDK action is a common driver of both mitotic cycles and endocycles. This is supported by the observation that overexpression of KRP reduced the level of endoreduplication as well as cell production (24, 147). From the increase of CYCD3 and CYCA3 expression in endoreduplicating maturing tomato tissues it was proposed that some D-type cyclins are involved in endoreduplication by promoting the G_1/S transition (61). However, this view cannot be generalized

toward all D-type cyclins, as overexpression of *Arabidopsis CYCD3;1* induced cell divisions in normally endoreduplicating trichomes. Furthermore, constitutive overexpression of CYCD3;1 prevented exit from the mitotic cycle and conferred hyperproliferation of leaf tissues while strongly inhibiting endoreduplication (28). In contrast, overexpression of the E2Fa/DP transcription factor, which stimulates genes involved in S phase, promotes both the mitotic cycle and endoreduplication (23). CDC6, which plays an essential role in the initiation of DNA replication in animals and yeast, is regulated by E2F transcription factors and upregulated in endoreduplicating cells (8, 101).

As mentioned above, endoreduplication is only initiated after the mitotic cell cycle is stopped (24, 37) and it correlates with loss of mitotic regulators. A further link is an activator of the anaphase-promoting complex, CCS52, the anaphase-promoting complex being responsible for the ubiquitin-related degradation of mitotic cyclins. Expression of *CCS52* was confined to dividing and endoreduplicating cells but was absent from cells with no cell-cycle activity. Antisense expression of *Medicago CCS52* reduced endoreduplication in hypocotyls, again suggesting that loss of mitotic cyclins is involved in the switch from mitotic cycles to endocycles (9). However, the upstream mechanisms influencing this mechanism are still unknown.

HORMONES AND THE CELL CYCLE

Studies spanning the past 50 years have shown the effects of plant hormones on cell proliferation. However, because most hormones also provoke morphogenetic effects, the cell-cycle consequences may be direct or part of the morphogenetic response.

Cytokinins and auxins are indispensable for maintaining undifferentiated cells in proliferation during in vitro culture and are the hormones most directly linked to cell proliferation. A detailed review by Jacqmard et al. (59) suggests that cytokinins have effects on the G_1/S and G_2/M transitions, as well as on progression through S phase. Cytokinins and brassinosteroids induce the expression of *CYCD3;1* (5, 22, 107), and the overexpression of *CYCD3;1* conferred cytokinin-autotrophic initiation and growth of *Arabidopsis* leaf calli, showing that in this system high levels of CYCD3;1 are sufficient to replace exogenous cytokinin (107). A mutation in the RPN12 subunit of the 26S proteasome conferred cytokinin insensitivity and a partially constitutive cytokinin response such as anthocyanin accumulation and inhibition of root growth and lateral root development. Interestingly, this mutation also conferred overexpression of *CYCD3;1* and *NIA1* (119). Therefore, it seems that pathways involving brassinosteroid and cytokinin signaling converge on *CYCD3;1* expression, which promotes entry into the mitotic cell cycle.

Tobacco BY2 cells do not require exogenous cytokinin, and zeatin-type cytokinins accumulate at the end of S phase and early M phase (102). Inhibition of isoprenoid biosynthesis at the G_2/M transition combined with rescue of the inhibition of mitosis by exogenous zeatin showed that this transition requires these

zeatin-type cytokinins (72), which might be linked to the nuclear localization reported for this type of cytokinin (27). G_1 progression was not as dependent on cytokinins in these highly proliferating cultures (73). Similarly, in a cytokinin-dependent *N. plumbaginifolia* cell suspension culture cells arrested in G_2 owing to the lack of cytokinins. Cytokinin induced dephosphorylation and hence activation of CDKA in these *N. plumbaginifolia* cells in G_2, as well as in tobacco pith cells grown in the presence of auxin alone (146). Cytokinin is therefore linked with CDK activation at the G_2/M boundary either by direct activation of a phosphatase or by downregulation of the WEE1 kinase.

Cell division is inhibited by abscisic acid. Abscisic acid induces the expression of the CDK inhibitor *KRP1/ICK1*, causing a decrease in the kinase activity associated with CDKA (138). Abscisic acid prevents tobacco BY2 cells from entering S phase but does not affect the progression through the other cell-cycle phases, consistent with the role of *KRP1/ICK1* in regulating CDKA/CYCD3 activity (138). Jasmonic acid application during G_1 prevented DNA synthesis, whereas jasmonic acid application during early S phase had only a minor effect on DNA synthesis but inhibited M phase progression (128). Ethylene treatment of cell suspensions provoked cell death at the G_2/M boundary (50).

CONCLUSION

As in other eukaryotes, the cell cycle is driven by CDK-controlled phosphorylation. In plants the core cell-cycle players have been identified, and it is clear that there is a larger number of genes than in animals, as well as completely new types of regulators including novel CDKs and E2F-like proteins. This complexity poses a challenge in the unravelling of the rules of this complex game. In particular, the mechanisms by which signal transduction cascades and developmental regulation impinges on the cell cycle are still largely unknown. However, considerable progress has been made, and the application of genomics tools is likely to lead to rapid further advances.

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