

The Plant Cell Cycle

An Overview

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Summary

This chapter reviews the basic features of plant cell proliferation. Although plant cell division seems to be largely similar to animals and fungi, there are a number of peculiarities that are perhaps related to their lifestyle and development. Initial comparisons of animal, fungal, and plant genomes suggest that the central cell division and growth machinery are largely similar but that some key regulatory molecules found in animals appear to be missing from plants. Also, many of the intracellular signaling molecules that coordinate growth during development and the proteins involved in their perception seem to be different between the groups. In some cases, this reflects true divergence in the underlying mechanism, but high rates of gene sequence divergence could explain other examples. However, what is undisputable is that plant and animal cells are highly adapted to different niches and that this is reflected in quite different behavior.

Key Words

Cell cycle; meristem; proliferation; cyclin; cyclin-dependent protein kinase; cdc2.

1. Plant Cells Differ From Animal Cells

1.1. Plant Cells Are Nonmotile

Perhaps one of the most significant differences between plant and animal cells, at least so far as the cell cycle is concerned, is the cell wall. Most animal cells lack a rigid extracellular coat and can move and change their shape relatively freely. Almost all higher plant cells are completely encased in a comparatively rigid carbohydrate-based wall that essentially eliminates cell migration and restricts shape change. Many of the other differences between plant and animal development, it could be argued, follow on from this basic cellular distinction. During plant development, cells are formed and

differentiate *in situ*, whereas animal cells can migrate relative to one another. The immobility imposed by the cell wall has profound consequences for how plants develop as multicellular organisms. Cellular migration is not an option, so differential proliferation and growth are the main mechanisms by which the plant body is generated. Cell proliferation occurs mainly in specialized regions called meristems, usually placed at the extremities of the plant body. Continuous proliferative activity within meristems provides cells for growth and also maintains the meristem.

As cells are displaced from the meristem, by proliferation and growth, they begin to differentiate and acquire defined cell fates. Cell fate is defined, at least in part, by signals coming from neighboring cells. The almost complete absence of cell migration, combined with the late positional definition of cell fate, means that plants, as a group, are not susceptible to systematic cancers. Indeed, even pathogen-induced cancers tend to be spatially very limited.

The meristematic mode of growth confers another notable characteristic on plant development, allowing the plant to continue to grow and develop throughout its life. Unless the meristem receives a signal to terminate growth and differentiate, it continues to grow and produce new tissues and organs. Animal development, on the other hand, occurs mainly during early embryogenesis, and later changes in body shape are mainly owing to growth of preexisting body parts.

1.2. Plant Cells Can Continue to Grow in the Postmitotic Phase

Plants seem to have partially compensated for the lack of cell mobility by evolving a remarkable ability to regulate their cell size. As in other eukaryotes, cell growth is somehow coupled to cell cycle progression, but many plant cells can grow extensively even when not actively dividing. There appear to be at least two distinct mechanisms for postmitotic growth, one involving endoreduplication of the genome, which therefore can be considered as coupled to cell cycle events, and the other, seemingly independent of cell cycle events, driven by vacuole expansion and under the control of specific plant growth regulators such as gibberellins.

2. Experimental Systems for Cell Cycle Studies in Plants

2.1. Cell Suspensions

A number of fast-growing cell suspensions have been developed over the years, but only a few are widely used. Perhaps the most popular has been the tobacco BY2 (Bright Yellow) line. This line grows as uniform filaments of cells that have lost the ability to differentiate but that can be synchronized to a very high degree for cell cycle studies (for review, *see* **ref. 1**). After release from aphidicolin-induced arrest in early S-phase, cells synchronously progress through G₂ and into mitosis. Up to 70% of cells can be in mitosis at the peak, making this the system of choice for cell cycle studies. The cell line can be readily transformed using *Agrobacterium* to transfer the DNA construct. A variety of well-characterized constitutive and inducible promoters in plant transformation vectors are available for driving gene expression. Codon-modified green fluorescent protein functions well as a tag for following the location and behavior of

proteins, or more traditional methods, such as indirect immunofluorescence, have been applied to BY2. Indeed, the large cell size, relatively low autofluorescence (for a plant cell), and optical clarity make it a useful model for cell biological studies.

A number of *Arabidopsis* cell lines have also been developed for similar studies, but these tend to grow more slowly, want to have irregular cell shape and size and higher autofluorescence than BY2. They also respond rather poorly to attempts at cell cycle synchronization, perhaps because aphidicolin is not completely reversible. However, a method has recently been published that produces populations enriched for G₁, S, and G₂ cells (2). Despite these disadvantages, *Arabidopsis* cell lines are likely to increase in popularity as a tool, if only to exploit the molecular and genetic tools developed by the various genome projects focused on this species.

2.2. Whole Plants

Genetic dissection of the cell cycle in fungi and flies provided many of the major insights into cell cycle regulation. This approach in plants is still in its early days, with cell cycle mutants resulting mainly as a byproduct of other screens. Mutants in the core cell cycle regulators, such as the cyclin-dependent kinases (CDKs), are conspicuous by their absence. Gene redundancy may be a factor—many genes, including the central cell cycle regulators, belong to large gene families with the potential for functional overlap, so knocking out a single gene may have little effect. However, systematic mutant screens are already very advanced in *Arabidopsis*, and large public collections are available from which insertional mutants produced by T-DNA insertion can be obtained. Lines containing multiple knockouts can be created by crossing, and these may uncover informative phenotypes. Another factor that reduces the likelihood of spotting phenotypes is the ability of plants to compensate for mild defects in cell division by increasing cell expansion and vice versa (3). Another possible reason could be gametophytic or early embryo lethality. Specific and comprehensive screens have been aimed at isolating such mutants (4–6), although the genes affected mostly remain to be identified.

Many of the same tools as used in cell suspensions can be applied to the study of cell cycle progression in plants. In addition, increasingly sophisticated imaging techniques are being developed to follow cell behavior in whole plants (7,8).

3. The Typical Plant Cell Cycle

3.1. The Cell and Microtubule Cycle

As in other eukaryotes, most plant cells sequentially pass through S-phase, when the genome is replicated, and M-phase or mitosis, when the genome is separated. Rapidly dividing meristematic cells might divide every 8–10 h, but most cells are much slower owing to increased Gap phases. The duration of both G₁- and G₂-phases can be increased, and, indeed, differentiated plant tissues can be a mix of cells arrested in either phase. Entry into S-phase has to be studied indirectly (i.e., by flow cytometry), but mitosis leads to cytological changes that reveal some of the interesting differences between plants and animals.

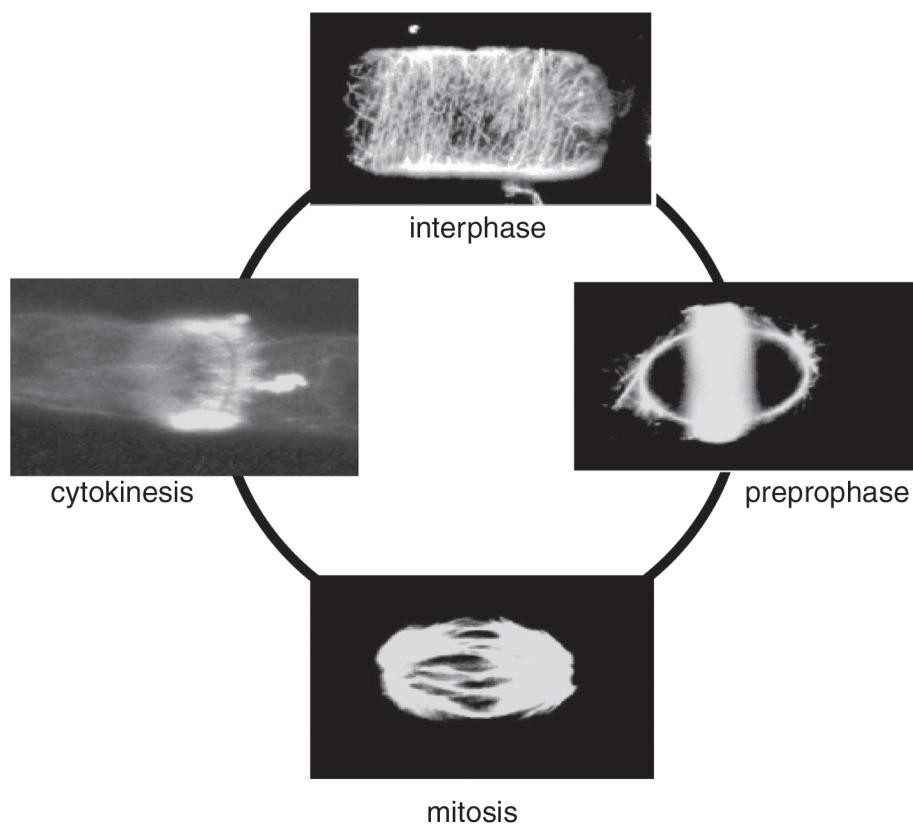


Fig. 1. The microtubule cycle in plant cells as revealed by indirect immunofluorescence. The interphase microtubule array, organized as bundles of microtubules in the cortex, gives way in G_2 to the preprophase band, which marks the site of cell division. The mitotic spindle has broad poles with several foci. The phragmoplast, involved in cell plate deposition during cytokinesis, is composed of a double ring of antiparallel microtubules.

3.1.1. The Microtubule Cycle: Entry Into Mitosis

Microtubules undergo dramatic reorganization during the cell cycle, as illustrated in **Fig. 1**. During interphase, the nucleus normally lies along one edge of the cell, but during the G_2 -phase it migrates to the site of nuclear division, typically to the center of the cell if the ensuing division is to give rise to two equal-sized daughters. Coincident with nuclear movement, the cell microtubules begin to rearrange. Many cells have predominantly cortical microtubules during interphase that are organized in short overlapping bundles. As such cells approach mitosis, a much more pronounced band of microtubules, called the preprophase band (PPB), develops in the area of the presumptive division plane. The site of the PPB accurately predicts the division plane, and the correlation has excited much interest over the years. The origin of the PPB is uncer-

tain, but cells that lack organized cortical interphase microtubules (owing to cell type or mutation) rarely have discrete PPBs. One possibility is that cortical microtubules move or collapse toward the presumptive division site and accumulate there, held by an unknown mechanism. Nuclear envelope breakdown is usually coincident with the late PPB and the early stages of spindle assembly.

3.1.2. Assembly of the Mitotic Spindle

Flowering plants completely lack centrioles, and, perhaps as a consequence, spindle assembly and organization appear distinctive. Centrioles play an important part in organizing spindle formation by providing a center for microtubule assembly in animals. In fungi, their place is taken by nuclear or spindle plaques, small multilayered structures that sit on or in the nuclear envelope, which also serve to organize microtubules. In the absence of discrete structured microtubule-organizing centers, spindle initiation appears to occur over the surface of the nucleus; as the spindle forms, the PPB is disassembled. The resulting spindle tends to have broad poles composed of numerous foci. Chromosomes condense and attach to the midzone of the spindle, presumably by mechanisms similar to or analogous to those described in other organisms.

3.1.3. The Phragmoplast: A Novel Plant-Specific Microtubule Array Required for Cell Division

Late in mitosis, another plant specific microtubule array, the phragmoplast, arises in the midzone of the spindle. This is composed of two sets of highly parallel sets of microtubules, each perpendicular to the plane of cell division and on opposite sides. These microtubules form an essential part of the mechanism by which the cross wall is laid down.

3.1.4. Reestablishment of Interphase Cellular Organization

As the nuclear envelope reforms around the nascent daughter nuclei, microtubules arise from the nuclear envelope and appear to spin out toward the cell cortex. At the cortex, these microtubules may be organized into the highly dynamic arrays typical of interphase cells.

4. Molecular Control of the Plant Cell Cycle

This section will briefly review our current understanding of key transitions during the cell cycle. Although the cycle is regulated at numerous stages, extracellular growth signals appear to act at two main points, G_1/S and G_2/M . The nature of these signals and their effects depends on the tissue and developmental stage. Auxin, for example, is the main positive proliferative signal during lateral root formation, but cytokinin is the dominant one in the shoot meristem. Both the signaling pathways and the way they affect the cell cycle are the subject of active research and debate, but some common principles are becoming apparent.

Most animal and fungal cells commit to a round of division at a defined point known as the restriction point or Start in G_1 -phase. After cells pass this stage, they are considered unable to respond to signals promoting alternative pathways such as those leading to differentiation. Plants also have a major control point during G_1 , reflected by the

fact that most differentiated cells arrest in G₁ in response to nutrient limitation or differentiation (reviewed in **ref. 9**). Although yeast and mammal experiments provided the initial insights in this area, studies on plants are interesting from the evolutionary angle, as well as being necessary to understand how the cell cycle responds during the development of a completely different multicellular organism.

4.1. The Cyclin D/E2F/RB Pathway and Entry Into the Cycle

Most differentiated cells, if they still contain a functional nucleus, can be induced by appropriate stimuli to dedifferentiate and reenter the cell cycle. Since one of the most important stimuli is wounding, this may be another adaptation to a sessile lifestyle, allowing repair of various types of damage. The nature of the wound signal is unknown, but exogenous plant growth substances are usually also required, particularly if cell proliferation is to be maintained.

The signal transduction pathway mediating cell cycle reentry is broadly analogous to that of mammalian cells, involving the transcriptional activation of cyclin D genes, inactivation of retinoblastoma (RB), activation of the transcription factor E2F, and production of proteins required for DNA replication (**Fig. 2**). Extracellular signals modulate the activity of an unknown signal cascade involving protein phosphorylation that leads to the synthesis of D-cyclins. Their associated kinase activity results in the phosphorylation and inhibition of an RB-like protein at the G₁/S boundary (**10**). The phosphorylated RB protein is thought to release transcription factors such as E2F that then promote the transcription of S-phase genes.

Plants contain an extensive array of cyclin D genes: genome analysis reveals that *Arabidopsis* has at least 10, as opposed to mammals, with 3. All members, in common with mammalian cyclin D proteins, contain a characteristic RB binding motif, LxCxE, near the C-terminus, and this has been shown to bind RB (**11–13**). Several plant viral proteins also bind RB, either via a typical LxCxE motif or via some other means, and perhaps modulate this pathway to ensure replication of their DNA. Experimental manipulation of the pathway using viral proteins or overexpression of RB can dramatically alter the potential of plant cells to proliferate (**14**).

Structural comparisons suggest that the D-cyclins fall into at least two major groups, the *cycD2* (three members) and the *cycD3* (three members), but there are also at least four orphans. The limited genetic data available suggest functional redundancy, in that an insertion knockout in the *cycD3;2* gene has no apparent phenotype (**15**) and no other mutations have been reported in this family. However, overexpression of various D-cyclins produces a variety of growth phenotypes, supporting the notion that they are limiting for growth. Expression of the *Arabidopsis cycD2;1* gene in tobacco causes faster but normal growth (**16**) while over-expression of *cycD3;1* in *Arabidopsis* causes abnormal growth and delayed differentiation in leaves (**17**).

Consistent with these observations, the cyclin D genes studied so far are under strict transcriptional and or translational control. In suspension cells, *CycD2;1* responds strongly to the availability of a carbon source (**18**) whereas *cycD3;1* responds to cytokinin, and expression of this gene can eliminate the requirement for cytokinin

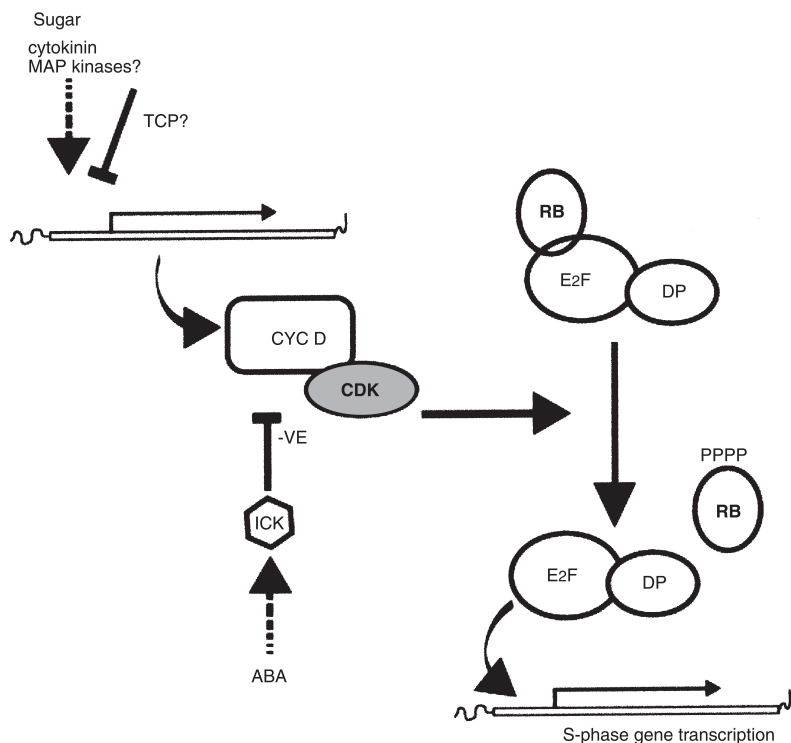


Fig. 2. The E2F/Rb/cyclin D pathway. Extracellular signals feed into the pathway by modulating the synthesis of components of CDK/cycD complexes. Positive signals such as cytokinin or sugar induce cycD transcription, whereas negative signals induce ICK transcription, probably via a signal transduction pathway involving mitogen-activated protein (MAP) kinases. In addition, regulatory transcription factors such as TCP also play a role, either directly or indirectly. The downstream part of the pathway seems largely similar to that in mammalian cells. CYCD, cyclin D; ICK, inhibitor cystine knot; TCP, Teosinte-branched/cycloidea/PCNA regulator.

in leaf explants (19). Most cyclin D genes are only highly expressed in proliferative regions of the plant, especially the meristem. Some are spatially restricted to certain regions: thus in *Arabidopsis* *cycD2;2* is expressed mainly in the lateral root (20), and in *Antirrhinum*, *cycD3A* is expressed only in the lateral organs (21). Moreover, the spatial domain of expression during floral morphogenesis seems to be regulated by *cycloidea*, a TCP-related transcription factor. The TCP gene family (Teosinte-branched/cycloidea/PCNA regulator) includes a number of developmental regulators, such as *teosinte-branched* from maize and *cincinata* from *Antirrhinum*, that have profound effects on plant morphology by differentially affecting growth within and between organs (22–24). TCP proteins can act as inhibitors of cell cycle gene expression by binding to *cis*-acting elements in their promoters (25).

Taken together, these results suggest that the different groups of *cycD* genes have different functions, probably operating in distinct pathways that tie cell proliferation to developmental or environmental responses. Thus, *cycD2* responds to sucrose availability and perhaps tailors plant growth rate to the current conditions, whereas *cycD3* acts downstream of pattern determinants, and overexpression has similar effects to that of an oncogene in mammals, leading to tissue disorganization.

As with cyclins, plants contain a complex family of cyclin-dependent protein kinases of which there are six classes, CDKA–CDKF. In animal cells, cyclin D proteins interact with a CDK variant, *cdk4*, but in plants the evidence suggests that the *p34^{cdc2}* ortholog, CDKA, is the main partner. CDKA also associates with mitotic cyclins, but it is the major cell cycle-related CDK expressed during G_1 . Indeed, CDKA protein levels and transcripts are fairly uniform throughout the cell cycle, and it is believed to play important roles from G_1 through to and within mitosis. Of course, CDK function is only partially characterized and some of the other variants may also play a role in G_1 (26).

In vivo CDKA/*cycD* kinase substrates have yet to be conclusively identified. Immunoprecipitated complexes can phosphorylate histone H1 but not Rb (27), although RB is used as a substrate by complexes assembled in insect cells (13).

4.2. S-Phase

If the E2F/RB pathway operates as in animals, then activated E2F must switch on a suite of genes whose products are required for DNA replication (28). A few such candidates have been identified and verified, including ribonucleotide reductase genes (29); proliferation cell nuclear antigen (PCNA; 29a,30), and *cdc6*, a component of the origin of replication complex (ORC; 31); CDC6 is synthesized in response to sucrose, probably by one of the E2F proteins (32). Combined microarray and bioinformatics surveys of the *Arabidopsis* genome suggest that there is a large number of other E2F targets, as judged by the presence of putative E2F binding sites in the 5' regions of the genes and cell cycle-regulated expression (33). However, E2F binding sites may depend on context, both genomic and developmental: the E2F sites in the PCNA promoter mediate gene activation in meristematic tissues but repression in differentiated tissues. Whether this means that E2F binds to some sites all the time, and is activation- or repression-regulated through accessory proteins such as RB, or that E2F proteins with different activities compete for the site is not clear. Some plant E2F factors act as activators, and others act as repressors, so both scenarios are possible.

The initiation of DNA replication is controlled by the pre-replication complex (RC), which contains the six proteins of the ORC and the minichromosome maintenance (MCM) proteins. These highly conserved proteins are sequentially recruited onto the OR prior to DNA replication, “licencing” the origin to commence replication. After replication is initiated, the pre-RC components are inactivated or eliminated from the complex, but the manner in which this occurs varies widely (34).

4.3. G_2/M

Mitotic entry occurs when *cdc2*-related protein kinases are activated. Overexpression of a dominant-negative form of CDKA that lacks kinase activity in tobacco plants led to plants that had fewer larger cells, whereas overexpression of

cyclin B was found to accelerate root growth in *Arabidopsis* (35), and local expression of cyclin A3 in tobacco induces local cell proliferation (36). Unfortunately, no details of the underlying mechanism are available for any of these examples, although one is tempted to assume a G_2 -based mechanism.

Cyclin B is tightly regulated at the transcriptional level during G_2 and early M-phase (37), in which it is probably rate-limiting for entry into M-phase. Transcriptional activation is mediated by small *cis*-elements in the 5' region of the gene that binds myosin binding protein (Myb)-like proteins, resembling c-MYB of animals (38). Plants appear to contain two classes of c-MYB-like proteins, one that activates and one that represses. The repressor MYB is present throughout the cell cycle, but the activator MYB is transcriptionally activated during G_2 and precedes cyclin B accumulation. Given their expression patterns and their ability to bind to the same site, an antagonistic mechanism has been proposed whereby the activating MYB displaces the inhibitor from the promoter, but this has yet to be proved *in vivo*. However, expression of the activator MYB gene in cells arrested in S-phase with a DNA synthesis inhibitor will induce cyclin B expression, suggesting that it is a limiting factor for G_2 /M-phase progression. Previously, c-MYB had been believed to activate genes only at the G_1 /S transition and was implicated in carcinogenesis, but recently a Myb protein has been shown to activate cyclin expression in *Drosophila* (39). This indicates that the mechanism controlling cyclin B transcription may be conserved between animals and plants. In plants, the activator MYB is only synthesized after S-phase is complete and presumably is under the control of a checkpoint-like signal pathway. The identity of this pathway is currently unknown.

At least some of the proteins involved in the spindle checkpoint are also conserved in plants (40), including MAD2. In maize, MAD2 is abundant at kinetochores during early mitosis but is barely detectable at kinetochores after the microtubules have attached (41). The existence of a spindle checkpoint mechanism in plants is further indicated by pharmacological studies. Treatment of synchronized plant cell cultures with microtubule-destabilizing drugs leads to a transient metaphase-like arrest, with highly condensed chromosomes scattered throughout the cell (42).

Mitotic progression also depends on the anaphase-promoting complex (APC)-mediated proteolysis of key regulatory proteins. The *Arabidopsis* genome contains genes homologous to the components of the APC. The N-terminal domains of both A and B cyclin confer cell cycle stage-specific instability on reporter proteins (43), suggesting that they contain functional destruction motifs. The proteasome inhibitor MG132, a peptide aldehyde that functions as a substrate analog, inhibits progression past metaphase by inhibiting the APC-dependent proteolysis of cohesion proteins responsible for sister-chromatid separation (44). Treatment of synchronized BY2 cell cultures with MG132 blocks cells in metaphase with elevated levels of CDK kinase activity and stabilized cyclins (43). Whether the metaphase arrest observed in plant cells is induced by a similar mechanism as in yeast and in animal cells (involving stabilization of chromatid cohesion proteins) is not known yet, but it seems clear that the mechanisms governing protein turnover during mitosis are largely conserved between plants and animals.

Plant cyclin B1 is degraded at the onset of anaphase and is stabilized during activation of the spindle checkpoint pathway by treatment with microtubule-disrupting drugs, similar to animal B-type cyclins (45). In contrast, plant cyclin B2 is degraded during mid-prophase, perhaps using a similar degradation mechanism to animal cyclin A. Thus cyclin B1 is not stabilized by activation of the spindle checkpoint, and its N-terminal destruction box contains multiple destruction box elements (46).

4.4. Mitotic Exit and Cytokinesis

Genetic dissection of early embryo development produced a rich harvest of mutants defective in different steps of cytokinesis and cell plate maturation. Cloning of the corresponding genes identified proteins that function during different events of vesicle trafficking like vesicle formation, transport, and fusion, revealing a highly controlled vesicle trafficking machinery implicated in plant cytokinesis (47). The phragmoplast consists of short bundles of antiparallel microtubules that are believed to mediate the delivery of Golgi-derived vesicles to the plane of division during the process of cell plate formation. Because the plus ends of phragmoplast microtubules (MTs) overlap at the equator, a plus-end-directed motor such as kinesin is believed to mediate vesicle transport, although no candidate has been identified from among the large family of kinesins described (47). Two proteins that associate with the cell plate in plant cells are related to the animal large GTPase dynamin, which is involved in endocytosis of synaptic vesicles. Phragmoplastin (48) and its *Arabidopsis* homolog *ADL1* (49) both seem to be involved in the formation of fusion tubes during the initial stage of cell plate formation.

The products of two genes, *Knoll E* and *KEULE*, have been found to mediate membrane fusion events concertedly during cytokinesis in the *Arabidopsis* embryo. *KNOLLE* encodes a cytokinesis-specific syntaxin expressed in vesicle-like structures during mitosis and at the phragmoplast (50). *KEULE* encodes a member of the Sec1 superfamily of proteins that are capable of inducing conformational changes in syntaxins and priming them for interaction with target proteins on vesicle membranes. *KEULE* has been shown to bind *KNOLLE* in vitro, and the synthetic lethality of *knolle/keule* double mutants indicates that the two proteins interact functionally in vivo (51,52). The precise function of the *KEULE/KNOLLE* complex is not known yet, but it might be involved in integrating cell cycle signals and transducing these to regulate the cytokinetic vesicle fusion machinery.

No homologs of polo-like kinases or aurora kinases, which are all involved in microtubule organization during mitosis and cytokinesis in animals and yeast, have been described in plants.

A mitogen-activated protein kinase (MAPK) kinase kinase (MAPKKK) known as NPK1 is located in the equatorial region of the phragmoplast. Overexpression of a kinase negative mutant form of the MAPKKK disrupts cytokinesis, suggesting a role in phragmoplast expansion toward the cell cortex (53). NPK1 was also found to interact with a tobacco MAPK kinase NtMEK1, which is known to interact with and activate the tobacco MAPK Ntf6 (54). Ntf6 is regulated in a cell cycle-specific manner, is

activated during anaphase and telophase, and also localizes to the phragmoplast (55). Thus a MAPK module composed of NPK1, NtMEK1, and Ntf6 is involved in plant cytokinesis, but downstream targets remain to be identified. NPK1 interacts with two kinesin-like proteins, NACK1 and NACK2, that stimulate NPK1 kinase activity and may be required for their localization (56). Mutations in the *Arabidopsis* homolog of NACK1, *HINKEL*, cause a defect in cytokinesis (57). The *SCD1* gene is also required for normal cell plate formation in *Arabidopsis*, and it too has homology to animal proteins involved in MAPK signal transduction (58).

4.5. Molecular Basis of Microtubule Organization in Plants

Microtubule organization is highly dynamic, changing in a characteristic pattern during the plant cell cycle. That microtubules play a crucial role in eukaryotic cell cycle progression is in no doubt—the role of plant-specific arrays such as the preprophase band and phragmoplast has been less clear until recently. Formal evidence that the phragmoplast plays a role in vesicle trafficking has been provided by mutations in the *PILZ* group of genes that eliminate microtubules and thereby cause a defect in cell plate deposition (59).

Although microtubule organizational changes have been extensively described, and many of the features seem to be specific to plant cells, the molecular basis of their organization remained obscure until very recently. Microtubule organizing center (MTOC) organization also seems to be radically different in plant cells. Cortical microtubules form parallel groups of overlapping tubules organized around the cell. The orientation of these bundles is highly dynamic, responding very quickly to external stimuli (60), supporting the idea that they control the orientation of cell growth.

Despite the lack of a recognizable MTOC, plants contain proteins similar to those normally found in the MTOC, but they behave differently. Thus, γ -tubulin, normally discretely localized in the MTOC of fungal and animal cells, is dispersed along interphase microtubules or is associated with other structures such as kinetochores and the nuclear envelope (61), supporting the idea of a dispersed MTOC activity that assumes various manifestation depending on the stage of the cell cycle (62). Recently we have shown that EB1, a microtubule plus and minus end-binding protein that plays a crucial role in regulating microtubule dynamics in animals ([63,64], accumulates at the poles of the spindle in surprisingly compact foci [64a]). EB1 also accumulates at the polar (minus) ends of the phragmoplast microtubules and as small dispersed foci at the ends of microtubule bundles in the cell cortex. Microtubules grow from and shrink back to these EB1 foci, providing direct evidence for the dispersed MTOC ideas of Mazia (62).

The molecular basis of coupling between the cell cycle and microtubule organization in animals seems to involve the phosphorylation of MAPs by cyclin B/CDK1, which then alters microtubule dynamics and perhaps organization. Although there have been several reports of association between CDK related proteins and the cytoskeleton (see third paragraph below), insight into the significance remains elusive. It remains

possible that CDK complexes are passive passengers, at least some of the time, and that other regulators mediate changes. A good candidate is PP2A: mutations in TON-NEAU2, which encodes for a regulatory subunit of PP2A, lead to the formation of very stunted plants whose cells cannot expand properly and seem to lack PPBs (65).

Although the plant and animal spindles have a similar overall organization and function, their structure and assembly differ. Plant spindles lack centrosomes, and the multiple spindle poles are usually less focused. Consistent with these observations, the most active MTOC in early mitosis is spread around the nuclear surface (66). Kinetochore may also serve as MTOCs in that microtubules appear to accumulate around kinetochores early in spindle formation (67), and at least two plant kinetochore proteins have homology to animal centrosome components. One of these is γ -tubulin (61,88), which is required for microtubule nucleation and is an important component of centrosomes in animals.

Microtubule motors and other associated proteins (MAPs) are required for spindle assembly and function, but many plant MAPs have been isolated that show little homology to animal MAPs. MAP65 proteins bind to subsets of interphase and mitotic microtubule arrays and bundle microtubules in vitro (69–71). These proteins belong to a small gene family that shares a remote ancestor with a yeast protein involved in cytokinesis (72) but have diversified in plants and perhaps acquired new functions. Many of the kinesin related proteins, whose function has been investigated, seem to have a role in phragmoplast formation rather than spindle movements. However, there is a large gene family of kinesin-related proteins in the *Arabidopsis* genome whose functions remain to be elucidated (73).

CDKA activity is required for normal entry into mitosis and the reorganization of mitotic microtubule arrays. Treatment of root tip cells with the cdk-specific inhibitor roscovitine disrupts mitotic spindle formation and leads to the formation of abnormal or monopolar spindles (55). Immunolocalization of CDKA and green fluorescent protein (GFP) fusion studies indicate dynamic interaction with the microtubules during mitosis (75,76). CDKA/GFP is found throughout the cytoplasm and nucleus during interphase but associates specifically with a subset of the PPB microtubules during late G₂. At the metaphase-to-anaphase transition, CDKA moves from the chromatin domain to the anaphase spindle, where it becomes microtubule-associated. The significance of microtubule association is not known, but immunogold localization suggests it is surprisingly intimate (73).

Furthermore, two mitotic plant cdk, cdkA and cdkB (75,76) and some plant cyclins, like ZmCycA1;1, ZmCycB1;1, and ZmCycB2;1 are present and are associated with the phragmoplast and the midline of cell division through anaphase, telophase, and cytokinesis (77). They might be involved in the regulation of MAPs like MAP65 or molecular motors like TPRK125 (78,79), which are also located at the midplane of cell division and contain potential cdc2 phosphorylation sites. Several other proteins including protein kinases have been localized to the growing cell plate in plants, but their precise functions remain to be determined. It seems likely that a complex regulatory network oversees the final step of mitosis, which ultimately severs a cell into two.

5. Modified Plant Cell Cycles

Subheading 4. described the typical cell cycle as observed in most meristematic and culture cells, but a number of variant types of cycle are found during normal plant development. These modified cell cycles are associated with specific types of differentiated cells whose genetic dissection has proved, almost incidentally, to be a useful source of cell cycle genes.

5.1. Endoreduplication

Cells normally alternate DNA replication with mitosis, thereby keeping the information content of their genome constant across generations. If successive rounds of DNA replication occur in the absence of mitosis, the DNA content of the cell doubles, and this is known as endoreduplication. Endoreduplication is characteristic of many different types of specialized plant cells such as xylem, large epidermal cells known as pavement cells, and, in some species, epidermal hair cells. There is a very strong correlation between DNA content and cell size in such cells (**80**), so perhaps endoreduplication provides a means of increasing cell size without the inconvenience of cell division (**81**). The correlation extends beyond specific cell types: polyploids tend to have larger cells than diploids in relation to their DNA levels. However, the reverse correlation is not so tight, and the final size of a particular cell is not always correlated with DNA content.

Although endoreduplication may use similar mechanisms for initiating DNA replication, mitosis is suppressed, and the mitosis-associated repression of replication reinitiation is suspended. Multiple rounds of DNA replication within a single cell cycle are usually prevented by the licensing system whereby the DNA RC somehow recognizes that replication has been completed. Therefore, perhaps the key step in the endocycle is reactivation of the DNA replication machinery without going through M. Reactivation is normally prevented by cyclin/CDK complex binding to the RC during G₂ and M, preventing its reactivation. During late M, the cyclin is degraded by the APC, and the RC is available again for reinitiation. In plants, endoreduplication requires either the prevention of cyclin B accumulation or its premature destruction. Activators of APC, such as the CDH1/FIZZY-related *ccs52* gene, fulfil this role (**82**). Overexpression of the cyclin B gene in trichomes changes an endocycle to a mitotic cycle (**83**), suggesting that suppression of the mitotic kinase is essential for the endocycle.

Arabidopsis mutants with altered levels of endoreduplication have been described by various labs, generally arising from two distinct types of screen that enrich for changes in cell size. Screens for altered trichome size and morphology have identified both positive and negative functions. The *SIAMESE* gene is required to suppress mitotic cell cycles, and its loss leads to ectopic cyclin B expression (**84**). Loss of *SPINDLY* (**85**) and several other genes (**86**) including *KAKTUS*, *RASTAFARI*, and *POLYCHOME* lead to ectopic endocycles in trichomes.

Screens that yield dwarf plants also tend to produce mutants with altered endocycles. A particularly interesting set of mutants in an unusual topoisomerase, *topo*

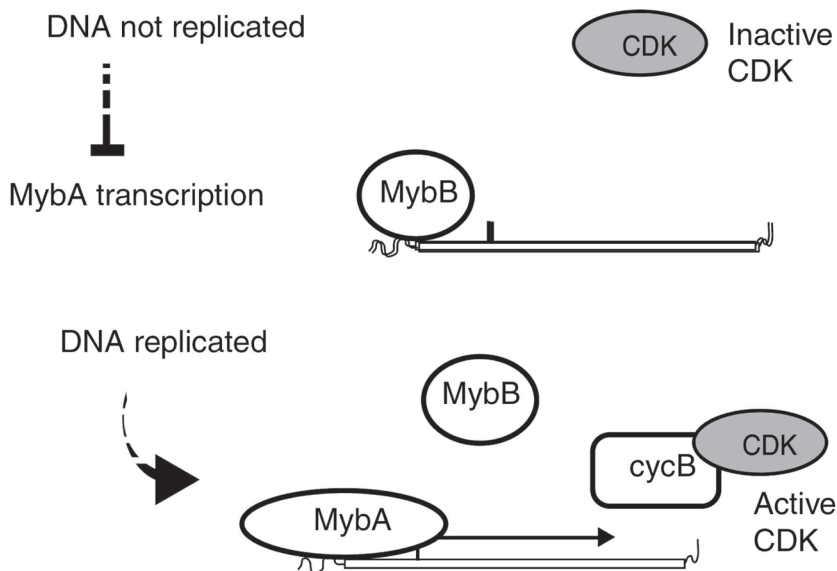


Fig. 3. A model for control of mitotic entry. Unreplicated DNA inhibits the production of activating MybA protein by an unknown mechanism, and inhibitory MybB occupies the promoter, preventing the transcription of M-phase-related genes, including cyclin. When DNA replication is complete, MybA is produced and displaces MybB from the promoter. Cyclin B is transcribed and activates cyclin-dependent kinase A (CDKA), allowing progression into mitosis.

VI, is required to allow cells to achieve very high levels of endoreduplication (87,88). Topo II normally resolves DNA strands after DNA replication in a process called decatenation. Topo II is required for the downstream separation of sister chromatids during mitosis, and mutations lead to G_2 arrest. This work indicates that the endocycle requires specific components in addition or instead of those required for the normal mitotic cycle.

The mechanism by which cell size and DNA content are linked should provide interesting insights into growth control in plants. Much of the increase in cell and organ size occurs outside the meristem in the postproliferative phase of the cell cycle, either as endoreduplication-associated cell growth or as vacuole-driven cell expansion. In the latter case, the increase in cell size is thought to be driven by primarily vacuole expansion.

5.2. Syncytial Cells

Plants contain a number of different cell types that have multiple nuclei, but perhaps the best studied is the nuclear endosperm. The endosperm arises as a result of double fertilization of a female gamete by two male nuclei. The resultant triploid tissue acts as nurse tissue for its sibling, the diploid embryo, facilitating nutrient transfer

in a manner roughly analogous to the mammalian placenta. The endosperm undergoes several rounds of rapid nuclear division and in many species cytokinesis is suppressed, producing a syncytium (89). This syncytial phase ends with simultaneous partitioning of the multinucleate cytoplasm into individual cells, a process referred to as cellularization (90). The molecular mechanism that suppresses cytokinesis is not well understood (91), but genetic dissection of endosperm development has revealed a large number of cell cycle-defective mutants. The genes thus identified include SMC genes (92), condensins (93), and tubulin assembly cofactors (94). Although some of these are specific for the endosperm, for example, the *SPATZLE* gene is specifically required for cellularization, many functions are also required for embryo development (90). The further study of endosperm development should thus provide useful genetic tools for dissecting the cell cycle generally as well as understanding its developmental modification

6. Conclusions

Plants provide a divergent system in which to study cell cycle regulation. The multicellular state is thought to have arisen independently in animals and plants, so the mechanisms that the two groups have evolved to ensure that cell growth and proliferation are subservient to the needs of the whole organism are likely to differ. One of the main challenges in the future will be to understand the pathways that control cell division during development. Cell cycle regulation also differs radically between different cell types within plants, revealing the extent to which cell division processes can be molded in response to development. The molecular processes underlying the unusual cell cycles should provide new insights into the regular cycle.

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