Cell Cycle Regulation in Plants

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Cell division plays a crucial role during all phases of plant development. Continuing organogenesis and plastic growth responses to a changing environment require precise spatial, temporal, and developmental regulation of cell division activity in meristems. The molecular analysis of cell division and its regulation in plants lags far behind such studies in yeast and animals. Since the cell theory was proposed by Schleiden and Schwann in 1838, many approaches have been taken to elucidate how cells divide, but insight into the molecular basis of division control developed only after the genetic analysis of cell division was initiated in yeast 25 years ago (Hartwell et al., 1970). Cell division cycle mutants in the yeasts Schizosaccharomyces pombe and Saccharomyces cerevisiae continue to provide a conceptual framework for analyzing gene interactions controlling the cellular processes leading to division. The convergence of genetic, biochemical, and molecular lines of inquiry during the last decade has shown that key cell-cycle regulators are universally conserved and has triggered the explosive growth of the field.

The complex network controlling growth in eukaryotes is hierarchically organized (Fig. 1). Regulatory pathways that communicate environmental constraints, such as nutrient availability, signals such as growth factors or hormones, as well as developmental cues control when and in which cells division occurs. In animals, genes such as ras and p53 are players in these pathways that activate the cell-cycle engine. This engine coordinates the various biochemical processes required for division. Although much is known about the biochemistry of these events, e.g. DNA synthesis, in most cases the question of how these metabolic pathways are activated remains unanswered. Cell-cycle regulation in animals and yeast has been extensively reviewed (Nurse, 1990; Forsburg and Nurse, 1991; Murray, 1992; Norbury and Nurse, 1992). This update will introduce the components of the eukaryotic cell-cycle machinery and their regulation, and then focus on progress in molecular dissection of cell-cycle regulation in plants.

THE CELL-CYCLE ENGINE AND ITS CONTROL

In all eukaryotes, the biochemical machine that controls progress through the cell cycle consists of a catalytic protein kinase and an activating cyclin subunit. This complex, but neither protein alone, has protein kinase activity. Stepwise changes of its activity and substrate specificity regulate progression through the cycle. To ensure that the reactions controlled by the engine are carried out to completion with sufficient accuracy and in the proper order, its activity is feedback regulated at checkpoints (Hartwell and Weinert, 1989). At such checkpoints its kinase activity becomes limiting for further progress until the feedback control network signals the completion of the dependent reactions, which then activates the kinase for passage through to the next checkpoint. These quantal changes of activity are mechanistically regulated by reversible phosphorylation of the engine components, by changes in subcellular localization of the complex, and by the rates of synthesis of limiting components.

CDKs

The proteins encoded by the S. cerevisiae CDC28 and the S. pombe cdc2+ genes are the prototypic CDKs required at both the G1/S and the G2/M transitions. In animals, CDK1 (=cdc2) is essential for the G2/M transition, whereas CDK2 and CDK3 are required for the G1/S transition (Heuvel and Harlow, 1993). These three CDKs share a short amino acid sequence, PSTAIRE. Several related kinases with homology but incomplete sequence identity to the PSTAIRE domain have been identified (Meyerson et al., 1992). Notwithstanding a function for CDKs in other processes not directly involved in cell-cycle regulation (Kaffman et al., 1994), one explanation for the evolution of the extensive CDK gene family in metazoans is increased opportunities for cell-cycle regulation during development.

CYCLINS

Cyclins were first identified in marine invertebrates as proteins whose levels oscillated during the cell cycle and that, when injected into frog oocytes, could induce meiosis. The observation that the S. pombe cdc13 gene, which encodes a cyclin, genetically interacts with cdc2 established a tight functional link between the components of the cell-cycle engine. Since then, an increasing number of cyclins have been identified in all eukaryotes studied (Lew and Reed, 1992). In S. cerevisiae, three cyclins (CLN1, CLN2, and CLN3) are expressed and presumably function in G1, two are expressed in

Abbreviation: CDK, cyclin-dependent kinase.

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for division occurs; and \( M \), in which the cellular contents are associate with the shaded area, which propels the cell through the \( S \) point during which the cell grows; \( S \), during which the nuclear genetic partitioned between two daughter cells. In animals and yeast, not yet been identified in plants. In animals, these \( G \) cyclins include ras, mitogen-activated protein kinases, and p53, function in \( G \) to activate \( G \) cyclins. Many of these crucial molecules have not yet been identified in plants. In animals, these \( G \) cyclins associate with CDK2 and CDK3. This results in a transient, rapid increase in protein kinase activity, indicated here by the width of the shaded area, which propels the cell through the START point and commits the cell to division. \( G \) cyclins are then inactivated but other cyclins, first \( A \) and then \( B \), take over the role of activating CDKs. After completion of DNA synthesis in plants, the transient formation of a prephase band is observed, which anticipates the position of the future cell wall. The cell cycle arrests at the \( G_2/M \) boundary due to limiting kinase activity of the cdcl/cyclin B complex. After the feedback control system, which monitors cell division, signals the faithful completion of \( G_2/M \) biosynthetic reactions, this complex is activated to initiate the visible cellular events ultimately leading to cytokinesis. The duration of cell-cycle phases is not drawn to scale.

**Figure 1.** The eukaryotic cell cycle is divided into four phases: \( G_1 \) during which the cell grows; \( S \) during which the nuclear genetic information is replicated; \( G_2 \) when further growth in preparation for division occurs; and \( M \), in which the cellular contents are partitioned between two daughter cells. In animals and yeast, signaling pathways that control cell division, key players of which include ras, mitogen-activated protein kinases, and p53, function in \( G \) to activate \( G \) cyclins. Many of these crucial molecules have not yet been identified in plants. In animals, these \( G \) cyclins associate with CDK2 and CDK3. This results in a transient, rapid increase in protein kinase activity, indicated here by the width of the shaded area, which propels the cell through the START point and commits the cell to division. \( G \) cyclins are then inactivated but other cyclins, first \( A \) and then \( B \), take over the role of activating CDKs. After completion of DNA synthesis in plants, the transient formation of a prephase band is observed, which anticipates the position of the future cell wall. The cell cycle arrests at the \( G_2/M \) boundary due to limiting kinase activity of the cdcl/cyclin B complex. After the feedback control system, which monitors cell division, signals the faithful completion of \( G_2/M \) biosynthetic reactions, this complex is activated to initiate the visible cellular events ultimately leading to cytokinesis. The duration of cell-cycle phases is not drawn to scale.

S phase (CLB5 and CLB6), and four are expressed in mitosis (CLB1, CLB2, CLB3, and CLB4). In animals, the \( C \), \( D \), and \( E \) class cyclins are expressed in \( G_1 \), cyclin A is expressed in the \( S \) and \( G_2 \) phases, and the \( B \) cyclins are expressed in the \( G_2 \) and early \( M \) phases. In most cells, the decision of whether to commit to division is made during \( G_1 \), and this was recently shown to be mediated by \( G_1 \) cyclins (Quelle et al., 1993). It is not understood how cyclins activate CDKs mechanistically. Cyclins are involved in determining the substrate specificity of CDKs (Peep et al., 1993) as well as targeting CDK activity to specific subcellular compartments during the cell cycle (Pines and Hunter, 1991).

**CELL DIVISION IN PLANTS**

Plant genes homologous to components of the cell-cycle engine have been cloned recently, but the function of these putative cell-cycle regulators has not yet been examined in plants. Therefore, at present it is unknown what function any of the genes described below has during plant cell-cycle progression.

**PLANT CDKs**

Putative CDK homologs have been cloned from pea (Feiler and Jacobs, 1990), alfalfa (Hirt et al., 1991), maize (Colasanti et al., 1991), Arabidopsis (Ferriera et al., 1991; Hirayama et al., 1991), soybean (Miao et al., 1993), and rice (Hashimoto et al., 1992). More than one gene was cloned from each of these plants and expressed in cdc2 or cdc28conditional mutants to examine whether it could rescue these yeast mutants. One alfalfa gene, cdc2MsA, passed this test. A second homolog, cdc2MsB, rescued only a S. cerevisiae cdc28 mutant that arrests at the \( G_1/S \) transition (Hirt et al., 1993). It is not apparent what the molecular basis for this is, because conserved amino acid motifs, including the PSTAIRE domain, are present in the cdc2MsB gene. Although both rice genes preserve the PSTAIRE motif, only one was able to rescue a cdc28 allele that arrests at the \( G_1/S \) transition (Hashimoto et al., 1992). Only one maize gene was tested, which was able to complement a cdc28 mutation (Colasanti et al., 1991). The two maize genes differ by seven amino acids, none of which represents a highly conserved residue. Both soybean genes were able to rescue a cdc28 mutant (Miao et al., 1993). Only one Arabidopsis gene, Atcdc2A, was able to rescue a cdc28 mutant, but in the second gene, Atcdc2B, the hallmark PSTAIRE motif occurred as PPTAIRE (Imajuku and et al., 1992). Likewise, in one of the PCR products amplified from pea, this motif appeared as PITAIRE (Feiler and Jacobs, 1991). Similar variations were observed in animal proteins related to the cdc2 kinase (Meyerson et al., 1992).

These results suggest that at least some of these plant genes can function as CDKs. However, it is not clear why several cdc2-related genes with conserved PSTAIRE motifs were unable to rescue yeast mutants. The recent discovery that a cyclin-CDK complex in yeast, PH080-PH085, functions in a regulatory pathway other than the control of cell division, yet PH085 contains a bona fide PSTAIRE motif (Kaffman et al., 1994), highlights the necessity of establishing the role of the above-mentioned genes during cell division in plants.

**PLANT CYCLINS**

Putative cyclins have been cloned from carrot, soybean, alfalfa, and Arabidopsis (Hata et al., 1991; Henley et al., 1992; Hirt et al., 1992). The soybean and Arabidopsis gene products promote nuclear envelope breakdown when the corresponding mRNAs are injected into Xenopus oocytes, indicating their functionality as mitotic cyclins. All plant cyclins described to date share homology to both A- and B-type cyclins within the cyclin box, an approximately 120-amino acid domain thought to mediate the protein-protein interaction with CDKs. It is unclear whether these plant cyclins play roles during the S phase, as reported for animal A-type cyclins, as well as during the \( G_2 \) and \( M \) phases, as shown for B-type cyclins. A-type cyclins have been found only in animals. Therefore, plant AB-cyclins may be early
members of an evolutionarily more divergent cyclin gene family. No G1 cyclin has yet been reported from plants.

REGULATION OF CELL DIVISION IN PLANTS

It is a safe assumption that the regulatory pathways that control meristem function ultimately target components of the cell-cycle engine. Therefore, the dissection of cell-cycle regulation in meristems was initiated with the analysis of the accumulation of cell-cycle engine components during development and changes thereof by environmental and hormonal signals. The expression of cdc2 homologs is higher in mitotically active tissues, such as floral buds, and low or undetectable in mature, nonproliferating organs, such as leaves (Colasanti et al., 1991; Ferreira et al., 1991; Hirt et al., 1991; Hashimoto et al., 1992; Miao et al., 1993). This analysis was taken to the cellular level in Arabidopsis (Martinez et al., 1992; Hemerly et al., 1993). Atcdc2 expression in all mitotically active meristems, but also in nonproliferating tissues such as the pericycle, from which lateral roots originate, or vascular cells responsible for secondary growth, indicates that cdc2 expression is a marker for the developmental competence to divide. When roots are treated with auxin, which stimulates cell division in a subset of pericycle cells that subsequently form lateral roots, normalized cdc2 mRNA levels in Arabidopsis do not increase, suggesting that RNA levels of the Atcdc2A kinase subunit of the cell-cycle engine are not limiting for meristem activity in this tissue. The developmental competence for division is not to be confused with totipotency, which is not developmentally programmed but is rather the capacity to reenter a proliferative state under artificial circumstances.

Whereas cdc2 mRNA expression correlates with the competence to divide, AB-type mitotic cyclin mRNA accumulates only in actively dividing cells. AB-type cyclin mRNA is expressed only in those cells of the floral meristem in Antirrhinum that are approaching mitosis, but is not observed in nonproliferating tissue (Fobert et al., 1994). However, accumulation of cyclin RNA only during this cell-cycle phase does not indicate that its abundance controls commitment to a new round of cell division, at least in the floral meristem, because this decision is made much earlier in the cycle, in G1. The temporal pattern of AB-type cyclin expression in plants parallels earlier observations of periodic cyclin accumulation (Evans et al., 1983). In animals and yeast, the observation of feed-forward and feed-back regulation of cyclin expression by cyclins has led to a model in which successive cyclin oscillations “help the cell cycle clock tick” (Amon et al., 1993; Edgar et al., 1994). This indicates that cyclin abundance limits progression in many types of cell cycle and that therefore control of cyclin expression is critical for the activation of the cell-cycle engine.

Regulation of the cell-cycle engine at other levels, such as by phosphorylation or differential subcellular localization, has not yet been reported in plants. The amino acid residues that are phosphorylated in yeast or animal CDKs are conserved in all cloned plant cdc2 homologs. Phosphorylation of Thr161 is essential for the activation of p34cdc2 in yeast and animals, and Tyr phosphorylation of Tyr15 is inhibitory for kinase activity in S. pombe and humans, but not in S. cerevisiae. The specialized kinases and phosphatases responsible for these modifications in animals and yeast have not yet been detected in plants. Recently, several homologs of mitogen-activated protein kinases were cloned from alfalfa, pea, and Arabidopsis (Duerr et al., 1993; Jonak et al., 1993; Mizoguchi et al., 1994). Mitogen-activated protein kinases function in growth-factor signal transduction upstream of the cell-cycle engine during G1, as well as downstream of it, in the M phase, to phosphorylate microtubule-associated proteins. Arabidopsis mitogen-activated protein kinase is activated in extracts from auxin-treated plants, suggesting that this class of kinase transduces mitogenic signals in plants.

Several aspects of cell division are unique to plants, but arguably the most important of these is how the plant deals with the constraints on morphogenesis imposed by the rigid cell wall. The rates and planes of cell division in all meristem cell layers are exquisitely coordinated to enable organogenesis. The signals and mechanisms by which the spatial coordinates of a cell’s environment and its developmental competence are translated into the selection of the correct plane of division are unknown at present. The earliest cytological indication of the position of the future cell wall appears transiently during late interphase with the preprophase band, a dense ring of cortical microtubules. A p34cdc2 gene product associates with the preprophase band in maize and onion, suggesting one interface where the cell-cycle engine and the pathways specifying the spatial execution of cytokinesis interact (Mineyuki et al., 1991; Colasanti et al., 1993). An exciting possibility is that a specific cyclin recruits p34 to the preprophase band.

GENETIC APPROACHES TO DISSECT GROWTH CONTROL

The paucity of conditional mutants makes genetic analysis of essential cell-cycle engine components in plants less feasible than in yeast, but a wealth of developmental and signal-transduction mutants, here restricted to a few from Arabidopsis, affected in the control of cell division remains to be exploited. Collectively, these mutants define the network of functions that, directly or indirectly, control cell-cycle engine activity. Alternatively, their gene products could be substrates of the cell-cycle kinase differentially expressed during development.

Several mutations identify genes that function as negative regulators of cell division activity. For example, cell division in apical meristems does not arrest in the dark, as it does in the wild type, after germination of de-etiolated (Chory et al., 1989) and constitutive photomorphogenic mutant seedlings (Deng et al., 1991). Vegetative, inflorescence, and floral meristems are enlarged in the clavata1 mutant due to increased cell numbers (Clark et al., 1993). In addition to other phenotypic changes, the loss of agamous function, a floral homoeotic gene, results in indeterminate growth of the inner floral whorl (Bowman et al., 1989). Three other mutants define pathways involved in cytokinesis. In gnom, the asymmetry of the first zygotic division is affected in such a way that the apical and basal daughter cells appear equal in size (Mayer et al., 1993). The lesion in monopteros becomes apparent at the octant stage of embryonic development, at which time
stereotypic divisions in the wild type that generate the cells that organize into the embryonic root do not occur (Berleth and Jürgens, 1993). The short-root mutant (Benfey et al., 1993) lacks a formative cell division that leads to the loss of the endodermal cell layer in the root.

**PERSPECTIVES**

In the next few years genetic analysis of all aspects of cell-cycle control in plants, the network controlling the activity of the cell division machinery, the cell-cycle engine itself, as well as the cellular functions controlled by it, will reveal the causal relationships governing growth. Complementing molecular, biochemical, and cytological analysis will begin to reveal the mechanisms, both general and species-specific, of cell division control in plants.

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**LITERATURE CITED**


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