

Cyclin-Dependent Protein Kinases and Their Regulators in Plants

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Abstract

A unique feature of plant cell cycle control is that quiescent meristematic and differentiated cells of plants are capable of re-entering the cell cycle. The activation of cell division and transitions between different phases of cell cycle are controlled by a family of cyclin-dependent serine/threonine protein kinases (CDKs). Post-translational regulation of CDKs plays an important role in the maintenance and activation of cell division, and thus controls plastic development of plant tissues. Here I review CDKs and cyclins in plants and describe the current view of CDK regulation by phosphorylation.

Introduction

The last few years have witnessed increased research interest into the mechanisms that control cell division in plants mainly due to the unique features of plant post-embryonic development; which is primarily controlled by cell division in meristems. Cell cycle is a sequential process that regulates division of eukaryotic cells, and the molecular mechanisms underlying the cell cycle are assumed to be well conserved from yeast to human. However, considering that plants need to accomplish a body plan throughout their life cycle in response to various internal and external signals, there should be some subtle differences from animal cells in the cell cycle machinery which controls the balance between cell division and differentiation.

In plants, quiescent meristematic and differentiated cells can re-enter the cell cycle (Fig. 1). For example, when *Rhizobia* induces the formation of root nodules on the roots of leguminous plants, the inner cortical cells, which are already differentiated, start to divide in response to nodulation (Nod) factors. In this case, the cortical cells are assumed to be arrested at the G1 phase, and re-enter the cell cycle with the stimulus of Nod factor (Yang *et al.*, 1994). In contrast, the pericycle root cells are known to be arrested at the G2 phase, and they produce new primordia for lateral roots by starting cell division under normal developmental processes (Blakely and Evans, 1979). It is well known that lateral root formation is influenced by environmental conditions and stimulated by a phytohormone, auxin, suggesting the plasticity of plant

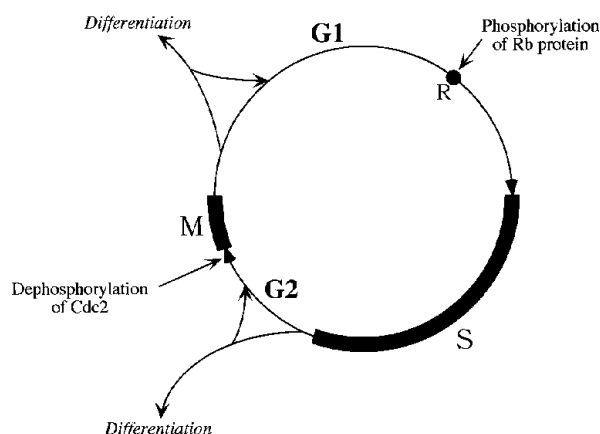


Fig. 1 Cell cycle regulation at G1-to-S phase and G2-to-M phase boundaries. Crucial events such as phosphorylation of Rb protein and dephosphorylation of Cdc2 are based on data from animals. Differentiated plant cells are usually arrested at the G1 or G2 phase of the cell cycle. R, restriction point.

cells in terms of cell division.

Studies in yeast, *Drosophila* and mammals have demonstrated that signaling pathways regulating cell cycle progression ultimately converge on the control of activity of cyclin-dependent serine/threonine protein kinases (CDKs) and their cyclin partners (Fig. 2). cDNAs encoding CDKs from many organisms have been isolated. A single major CDK has been identified in the fission yeast *Schizosaccharomyces pombe* (CDC2) and in the budding yeast *Saccharomyces cerevisiae* (CDC28) (Hindley and Phear, 1984; Lőrincz and Reed, 1984). However, the growing list of CDKs in human cells suggests that, during development, each CDK in metazoans

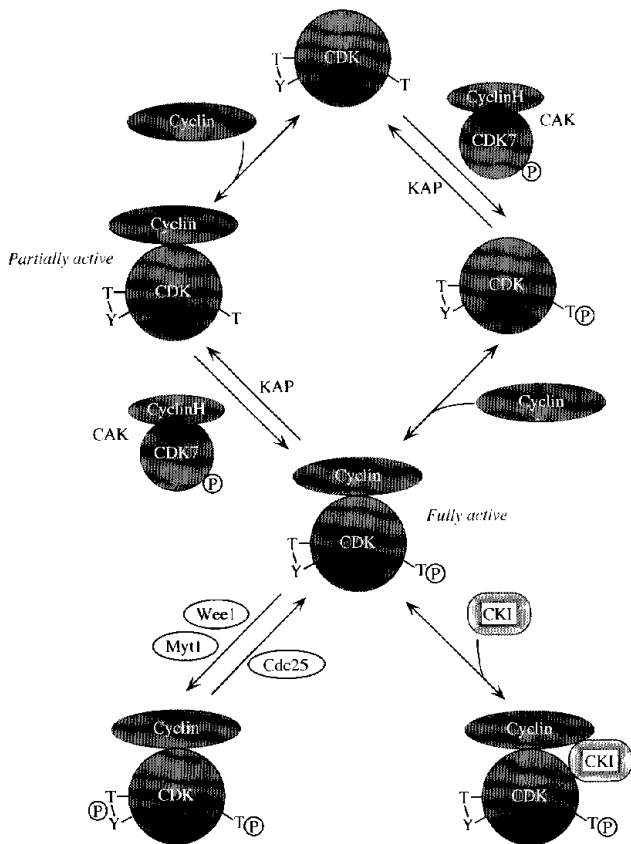


Fig. 2 Principles of CDK regulation. CDK is fully activated by binding to cyclins and by phosphorylation with CDK-activating kinase (CAK). The order of cyclin binding and phosphorylation in this activation process is different among various CDKs. CAK is also assumed to be activated by phosphorylation. The phosphorylated sites are dephosphorylated by KAP phosphatase. On the other hand, CDK activity is inhibited by phosphorylation of the other sites by Wee1 and Myt1 kinases, and dephosphorylation of these sites by Cdc25 phosphatase results in activation of CDKs and promotes progression through G2 to M phase. CDK inhibitors (CKI) also inhibit CDK activities post-translationally.

plays a specific role at a specific time in the cell cycle. Each CDK interacts with a specific subset of cyclins, and the size of this subset varies among CDKs. For example, CDC28 can associate with many different cyclins, whereas human CDC2 interacts with relatively few (for a review, see Nigg, 1995). In vertebrates, at least eight types of cyclins have been identified; including mitotic cyclins (cyclin A and B), G1 cyclins (cyclin D, E, G) and three groups of cyclin-related proteins (cyclin C, F, H). A short conserved amino acid sequence, PSTAIRE, in CDKs is responsible for the binding of cyclins, which activate CDKs by changing the conformation at the catalytic site (Jeffrey *et al.*,

1995; Morgan, 1996). They also function in the targeting of CDKs to specific substrates or subcellular locations (Hoffmann *et al.*, 1993; Peeper *et al.*, 1993; Dynlacht *et al.*, 1994).

The function of cyclins is regulated at a transcriptional level as well as by proteolytic pathways. It is well known that PEST sequences and destruction boxes mediate the degradation of cyclins during defined phases of the cell cycle or in response to internal or external signals. In yeast, the degradation of G1/S phase cyclins Cln1 and Cln2 is mediated by an E3 ubiquitin ligase complex consisting of the SKP1-Cdc53/cullin-Grr1 proteins (SCF^{Grr1}) (for a review, see Tyers and Williams, 1999). The N-terminus of CDC53 binds to the SKP1 chaperonin / adapter protein which then interacts with F-box proteins that carry a cyclin-F homology domain. Grr1 is one of the F-box proteins that functions as a receptor by recruiting target cyclins to E2 ubiquitin carriers and E3 ubiquitin ligases. The SCF-complexes appear to be active throughout the cell cycle, although their activity seems to be controlled by post-translational modification.

The decision whether the cell cycle progresses or not is dependent on the signal controlling transition through the "restriction point" (Fig. 1). The retinoblastoma (Rb) proteins play an important role at this point, because once Rb proteins are hyperphosphorylated by CDK and cyclin D/E complexes, they are released from the transcription factor E2F which then induces the gene expression involved in transition from G1 to S phase (Dyson, 1998). Since the transcript level of cyclin is controlled by internal signals in animals, the restriction point is a timing at which CDKs communicate with environmental conditions and hormonal signals. On the other hand, the transition from G2 to M phase is tightly regulated by phosphorylation of Cdc2. The inhibitory phosphorylation of Cdc2 results in retention of cells at the G2 phase, and dephosphorylation of specific amino acid residues induces the sequential process of mitosis (Fig. 1). Therefore, post-translational regulation of CDKs is essential for a proper operation of cell cycle in both G1 and G2 phases.

Cyclin-dependent protein kinases in plants

As in animals, plants also express various types of CDK, and multiple genes for CDKs have been found in *Arabidopsis* (Ferreira *et al.*, 1991; Hirayama *et al.*, 1991), alfalfa (Hirt *et al.*, 1991, 1993), rice (Hata, 1991; Hashimoto *et al.*, 1992; Kidou *et al.*, 1994), soybean (Miao *et al.*, 1993), maize (Colasanti *et al.*, 1991), and *Antirrhinum* (Fobert *et al.*, 1994). Recently, Fobert *et al.* (1996) isolated

four *cdc2*-related genes from *Antirrhinum* and Magyar *et al.* (1997) described four homologs of *cdc2* in alfalfa, in addition to *cdc2MsA* and *cdc2MsB* that had been isolated previously (Hirt *et al.*, 1991, 1993). These findings suggest that the division of plant cells might be regulated by different sets of CDK/cyclin pairs at each stage of the cell cycle, and that division is not controlled by a single major CDK as in the case of yeast (Doerner, 1994; Ferreira *et al.*, 1994b; Murray, 1994; Doonan and Fobert, 1997).

Genes for four different CDKs have been isolated from rice: *cdc2Os1*, *cdc2Os2* (Hashimoto *et al.*, 1992) and *cdc2Os3* (Kidou *et al.*, 1994; Umeda *et al.*, 1999b) are homologs of *cdc2*; and R2 (Hata, 1991), which is similar to the gene for a CDK-activating kinase (CAK) that will be described below. The deduced amino acid sequence of Cdc2Os3 showed that it is distinct from proteins in the CDC2/CDC28 family (Kidou *et al.*, 1994), while *cdc2Os1* and *cdc2Os2* are closely related to the homologs of *cdc2* that have been isolated from various organisms (Hashimoto *et al.*, 1992). Cdc2Os3 has a PSTAIRE domain that is not conserved in amino acid sequence, as shown in Table 1. Other CDKs carrying non-PSTAIRE sequences have also been reported in *Antirrhinum*, alfalfa and *Arabidopsis* (Fobert *et al.*, 1996; Magyar *et al.*, 1997; Segers *et al.*, 1996). One of the features of these non-PSTAIRE CDKs is that they are unable to complement the *cdc28* mutation when expressed in temperature-sensitive mutant of budding yeast. In contrast, the PSTAIRE CDKs could at least partially complement the mutation, thus they are functionally homologous to yeast CDC28 (Ferreira *et al.*, 1991; Hirayama *et al.*, 1991; Hashimoto *et al.*, 1992; Hirt *et al.*, 1991, 1993; Fobert *et al.*, 1996; Colasanti *et al.*, 1991; Miao *et al.*, 1993). Since the PSTAIRE CDKs are assumed to form active CDK/cyclin complexes in yeast cells, one can speculate that non-PSTAIRE CDKs might have distinct functions in the cell cycle and form active complexes with particular cyclins specific to plants. Indeed, plants have several specific subclasses of cyclins as discussed below.

Expression of plant CDKs during the cell cycle

The most striking difference between PSTAIRE and non-PSTAIRE CDKs is the expression pattern during the cell cycle. A consensus has recently emerged indicating that every PSTAIRE-type CDK shows an constitutive expression throughout the cell

cycle, indicating that they function by interacting with different cyclins in each phase of the cell cycle (Table 1). This supports the notion that PSTAIRE CDKs are functional homologs of CDC28, which is the sole CDK that directly controls the cell cycle in budding yeast. On the other hand, non-PSTAIRE CDKs are classified into three groups based on the expression pattern. The first group consists of CDKs that show constitutive transcript levels and are distantly related to yeast CDC2/CDC28 based on amino acid sequence (Table 1). For example, rice R2 is a homolog of CDK-activating kinase (Hata, 1991), Alfalfa Cdc2MsC has 53% identity with the human cholinesterase-related cell division control protein (CHED), and Alfalfa Cdc2MsE showed only 33 to 40% identity with plant and animal Cdc2 and CDK kinases (Magyar *et al.*, 1997). The other two groups of non-PSTAIRE CDKs are distinct from other CDKs because their transcripts accumulate at a particular phase of the cell cycle, namely during the G2-to-M phase or during the S-to-M phase (Table 1).

Magyar *et al.* (1997) examined the expression period of Alfalfa CDKs by using synchronized suspension cultured cells. The results showed that

Table 1. Amino acid sequences in the PSTAIRE region of plant CDKs. Amino acids that are conserved in the PSTAIRE region are shown in bold letters. Cdc2Os1-3 and R2, CDKs of *Oryza sativa*; Amcdc2a-d, CDKs of *Antirrhinum majus*; Cdc2aAt and Cdc2bAt, CDKs of *Arabidopsis thaliana*; Cdc2MsA-F, CDKs of *Medicago sativa*.

(A) CDKs expressed throughout the cell cycle	
1) PSTAIRE type	
Cdc2Os1*	EGV PSTAIRE ISLLKE
2) non-PSTAIRE type	
R2	EGVN F TALREIKLLKE
Cdc2MsC	EG F P I TALREIKILKK
Cdc2MsE	DGV S P T AIREIMLLRE
(B) CDKs expressed during the S-to-M phase	
AmCdc2c	EGV P P T ALREVSLQLM
Cdc2MsD	EGV P P T ALREVSLQLM
Cdc2bAt	EG I P P TALREISLLQM
(C) CDKs expressed during the G2-to-M phase	
Cdc2Os3	EGV P P T ALREVSLLRM
AmCdc2d	EGV P P T TLREVSLLRM
Cdc2MsF	EGV P P T TLREVSIILRM

*Cdc2Os2, AmCdc2a, AmCdc2b, Cdc2MsA, Cdc2MsB and Cdc2aAt have identical sequence in the corresponding region.

transcripts of *cdc2MsD* were abundant at the G2/M phase and were also detected just after alfalfa cells were released from arrest by aphidicolin, indicating that *cdc2MsD* was expressed for a longer period during S to M phase. In contrast, mRNA of *cdc2MsF* was detected only from the G2 to the M phase. Similarly, several CDKs from other plants can be classified into those with expression pattern specified for G2-to-M phase or S-to-M phase (Table 1). Unlike in Alfalfa, synchronization of rice or *Antirrhinum* cells is difficult because of the low efficiency of synchronization. In such cases, *in situ* hybridization of meristematic tissues is a powerful tool for studies of cell cycle-dependent expression of genes (Fobert *et al.*, 1994, 1996; Kouchi *et al.*, 1995; Umeda *et al.*, 1999a, b).

We have recently analyzed the levels of transcript of genes for rice CDKs by *in situ* hybridization of root sections (Umeda *et al.*, 1999b). While transcripts of *cdc2Os1*, *cdc2Os2* and *R2* were detected uniformly in dividing cells of roots, the distribution of *cdc2Os3* showed a patchy pattern in the dividing region of the root apex. The same pattern was also observed in the region near the shoot meristem and in the primordia for lateral root formation. Counterstaining of sections with DAPI indicated that almost all cells with mitotic nuclei contained *cdc2Os3* transcripts, while cells forming cell plates had trace levels of the transcripts. In double-labeling experiments with probes specific for transcripts of a gene for histone H4 and *cdc2Os3*, signals did not overlap, an indication that expression of *cdc2Os3* did not extend to the S phase. Thus, transcripts of *cdc2Os3* appeared to be abundant from the G2 to M phase but almost disappeared when cells had completed mitosis at telophase. These results suggested that the Cdc2Os3 protein might function in mitosis.

Rice *cdc2Os3* encodes a PPTALRE sequence which is similar to those of *Antirrhinum AmCdc2c*, Alfalfa *Cdc2MsD* and *Arabidopsis Cdc2bAt* (Table 1). However, when Cdc2Os3 was compared with the other non-PSTAIRE CDKs in the whole region, it was located close to *Antirrhinum Amcdc2d* and Alfalfa *Cdc2MsF* rather than to *AmCdc2c*, *Cdc2MsD* or *Cdc2bAt*. Transcripts of *cdc2s* belonging to the group including *cdc2Os3* were abundant from the G2 to the M phase. On the other hand, *Amcdc2c* is expressed from mid S phase to early M phase (Fobert *et al.*, 1996), and *cdc2bAt* is preferentially expressed during the S and G2 phases (Segers *et al.*, 1996). As discussed above, transcripts of *cdc2MsD* are abundant at the G2/M phase (Magyar *et al.*, 1997). Therefore, we propose that the products of *cdc2Os3*, *Amcdc2d* and *cdc2MsF* form a distinct subclass of non-PSTAIRE CDKs which are pre-

ferentially expressed from the G2 to the M phase (Table 1). Our results also indicated that domains other than the PSTAIRE region are important for the function of non-PSTAIRE CDKs in distinct cell cycle phases.

Plant mitotic cyclins and their expression patterns

A- and B-type cyclins are mitotic cyclins required for progression from the G2 to M phase. A common feature between these two types of cyclins is that they have a central region encompassing approximately 250 amino acids, called the cyclin box (Nugent *et al.*, 1991). The cyclin box is sufficient for binding to and activating CDKs (Brown *et al.*, 1995; Jeffrey *et al.*, 1995; Bazan, 1996). A- and B-type cyclins have another motif called the destruction box at the N-terminal region, which is composed of nine amino acids and is involved in the degradation of cyclins during mitosis (Glutzer *et al.*, 1991). In plants, many cDNAs encoding mitotic cyclins have been isolated from various plant species (for a review, see Renaudin *et al.*, 1998). Based on the amino acid similarity, A- and B-type cyclins have been further classified into small groups, namely CycA1-A3 and CycB1-B2 (Renaudin *et al.*, 1996). The growing list of plant mitotic cyclins suggests that the progression from the G2 to M phase is controlled by CDKs in association with several types of cyclins.

Experiments using synchronized suspension cells and *in situ* hybridization with meristematic tissues indicated that expression patterns of plant mitotic cyclins are divergent. Tobacco CycA1 genes such as *NtcycA19*, *Ntcyc25* and *Ntcyc27* are known to be expressed from the mid or late S phase to M phase (Setiady *et al.*, 1995; Reichheld *et al.*, 1996). In contrast, rice *cycA1;1* is preferentially expressed in a more restricted period during the G2 to M phase, and the transcripts almost disappear in the metaphase of mitosis (Umeda *et al.*, 1999a). We have recently investigated the transcripts of two B2-type cyclins of rice, namely *cycB2;1* and *cycB2;2*, by *in situ* hybridization and counterstaining with DAPI (Umeda *et al.*, 1999a). About 56% of the cells expressing *cycB2;1* showed mitotic nuclei, in which 10% were in anaphase represented by two daughter chromosomes. The proportion of cells in each phase of mitosis was almost the same between *cycB2;1* and *cycB2;2*, suggesting that both cyclins were expressed until the end of mitosis. When counted for each mitotic phase, over 90% of the prophase and metaphase cells contained transcripts of

cycB2;1 and *cycB2;2*, while about 70% of the cells with two daughter chromosomes showed signals for their transcripts. Therefore, it seems that the transcript levels of these B2-type cyclins were reduced during the progression of the anaphase. These cyclin and *cdc2Os3* genes were expressed in a period overlapping with each other in the cell cycle. Therefore, Cdc2Os3 may interact with such B2-type cyclins and be involved in the control of mitosis. In fact, we have recently demonstrated that Cdc2Os3 specifically binds to one of the two B2-type cyclins *in vitro* (unpublished results).

Ferreira *et al.* (1994a) investigated whether the expression of *Arabidopsis cycB1;1* is dependent on phytohormone action. The promoter of *cycB1;1* was fused to β -glucuronidase gene, and the construct was transformed into tobacco cells. In protoplasts, an increase in *cycB1;1* expression was observed when both auxin and cytokinin were included in the culture medium. However, treatment with either auxin or cytokinin did not cause any increase in the gene expression. This indicates that the expression of *cycB1;1* is induced when cell division is activated, but is not responsive to phytohormones themselves. In general, transcripts of genes for mitotic cyclins are abundantly present in tissues with high cell division activity. Sauter *et al.* (1995) have shown that the transcript levels of rice *cycB2;1* and *cycB2;2* are increased by gibberellin treatment in the intercalary meristem of deepwater rice internodes. This suggests that induction of mitosis by gibberellin may induce a rapid growth of internodes under submergence conditions which in turn promotes ethylene synthesis and subsequent reduction and increase in the level of abscisic acid and gibberellin, respectively (Hoffmann-Benning and Kende, 1992).

Post-translational regulation of CDKs

It is known in yeast and animals that activation of CDKs is controlled by phosphorylation, as well as by binding to cyclins (for review, see Morgan, 1995). Most CDKs have a conserved phosphorylation site within the T-loop of kinase subdomain

VIII. For example, residue 160 of human CDK2 is threonine and phosphorylation of this residue, which changes the structure of the T-loop, is required to allow substrates to gain access to the entrance of the active-site cleft (De Bondt *et al.*, 1993; Fisher, 1997; Jeffrey *et al.*, 1995; Morgan, 1996; Russo *et al.*, 1996). Phosphorylation of the threonine residue is catalyzed by a CDK-activating kinase (CAK) (Fisher and Morgan, 1994; Labbé *et al.*, 1994) (Fig. 2). In contrast, CDK activities are inhibited by phosphorylation of threonine 14 and tyrosine 15 by Wee1 and Myt1 kinases (Russell and Nurse, 1987; Lundgren *et al.*, 1991; Parker *et al.*, 1992; Mueller *et al.*, 1995; Liu *et al.*, 1997). These phosphorylation sites are buried beneath a loop structure, and cyclin binding induces a conformational change that makes these residues accessible for phosphorylation (De Bondt *et al.*, 1993). It is well known that dephosphorylation of these sites by dual specificity phosphatase, Cdc25, activates CDKs and triggers the G2-to-M phase transition (Kumagai and Dunphy, 1991). Therefore, both CAK and Cdc25 are essentially required for the full activation of CDKs (Fig. 2). In addition, CDK activities are inhibited by interaction with CDK inhibitors (Fig. 2). The mammalian CDK inhibitors can be grouped into two families, p21^{Cip1}/p27^{Kip1} and INK4a (for a review, see Harper and Elledge, 1996).

Purification of CAKs from starfish, *Xenopus* and mammals identified a heterotrimeric complex composed of the catalytic subunit Cdk7/p40^{MO15}, the regulatory subunit cyclin H, and the assembly factor MAT1 (Devault *et al.*, 1995; Fesquet *et al.*, 1993; Fisher and Morgan, 1994; Fisher, *et al.*, 1995; Mäkelä *et al.*, 1994; Poon *et al.*, 1993; Solomon *et al.*, 1993) (Table 2). The Cdk7-cyclin H complex has also been identified in the general transcription factor TFIIF, which is involved both in RNA polymerase II-dependent transcription and in nucleotide-excision repair of DNA (Nigg, 1996; Roy *et al.*, 1994; Shiekhattar *et al.*, 1995; Serizawa *et al.*, 1995; Seroz *et al.*, 1995). Cdk7-cyclin H phosphorylates not only CDKs but also the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II (Rossignol *et al.*, 1997; Roy *et al.*,

Table 2. Protein kinases regulating activation of CDKs and basal transcription.

	<i>Arabidopsis</i>	Rice	Vertebrates	<i>Sch. pombe</i>	<i>S. cerevisiae</i>	
Catalytic subunit	Cak1At	R2	Cdk7/p40 ^{MO15}	Crk1/Mop1	Kin28	Cak1/Civ1
Cyclin subunit	?	Cyclin H	Cyclin H	Mcs2	Ccl1	None
Assembly subunit	?	?	MAT1	?	Rig2	None
CAK activity	Yes	Yes	Yes	Yes	No	Yes
CTD-kinase activity	No	Yes	Yes	Yes	Yes	No

1994; Shiekhattar *et al.*, 1995; Serizawa *et al.*, 1995; Seroz *et al.*, 1995; Tassan *et al.*, 1995; Yankulov and Bentley, 1997). Phosphorylation of the CTD is required for the release of RNA polymerase II from the preinitiation complex and the initiation of transcription (O'Brien *et al.*, 1994; Yankulov *et al.*, 1995, 1996). This indicates that Cdk7/p40^{M015} plays an additional role in transcription.

In *Schizosaccharomyces pombe*, Crk1/Mop1, which is closely related to Cdk7/p40^{M015}, associates with cyclin Mcs2 and phosphorylates both Cdc2 and CTD *in vitro* (Buck *et al.*, 1995; Damagnez *et al.*, 1995) (Table 2). In contrast, in *Saccharomyces cerevisiae*, Kin28, the closest relative to Cdk7/p40^{M015}, is a component of TFIIF that has CTD-kinase activity but does not exhibit the activity of a CDK-activating kinase (CAK) (Cismowski *et al.*, 1995; Feaver *et al.*, 1994) (Table 2). An additional protein kinase, designated Cak1/Civ1, has been identified that has CAK activity *in vivo*, but it displays less than 25% sequence identity to its closest homologues (Espinoza *et al.*, 1996; Kaldis *et al.*, 1996; Thuret *et al.*, 1996). Unlike vertebrate and fission yeast CAKs, Cak1/Civ1 is known to be active as monomer (Table 2). Therefore, in budding yeast two distinct protein kinases regulate the activation of CDKs and basal transcription. Recently, Larochelle *et al.* (1997) created null and temperature-sensitive mutations in the *cdk7* gene of *Drosophila* and demonstrated that Cdk7 is essential for mitosis and for CAK activity *in vivo*. Therefore, it is possible that there might be a fundamental difference in terms of the functions of CAKs between metazoans (or fission yeast) and budding yeast.

CDK-activating kinases in plants

A cDNA encoding a CAK homolog of *Arabidopsis* (*cak1At*) has been isolated from a cDNA expression library by complementation of a budding yeast *cak* mutant (Umeda *et al.*, 1998). Although Cak1At shows the closest similarity to the Cdk7/p40^{M015} kinase family within specific domains, other CDK regions are less conserved in Cak1At which therefore cannot be classified as part of the group of CDC2/CDC28 and Cdk7/p40^{M015} kinases. We found that Cak1At is capable to rescue the temperature-sensitivity of both fission and budding yeast *cak* mutants. Since CAKs of budding and fission yeasts are completely different from each other as described above, *Arabidopsis* Cak1At may function in a unique way in activation of CDKs. Unlike Cdk7/p40^{M015}, Cak1At could phosphorylate and activate human CDK2 *in vitro*, but did not show

any kinase activity on *Arabidopsis* CTD (Umeda *et al.*, 1998). Although this feature resembles that of budding yeast Cak1/Civ1, Cak1At showed no significant sequence similarity to Cak1/Civ1 (Table 2).

In rice, the amino acid sequence of CDK/CAK homolog R2 (Hata, 1991) is very similar to Kin28 of budding yeast (Table 2). In fact, R2 is included into the same cluster as Cdk7/p40^{M015} in the phylogenetic tree. Overexpression of R2 suppressed the *cak* mutation in budding yeast but not in fission yeast (Yamaguchi *et al.*, 1998). R2 functions as a CAK to phosphorylate both CDK and CTD, as reported for CAKs of metazoans and fission yeast, thus it appears to be the first CTD-kinase identified in plants. We found the R2 protein mainly in protein complexes of approximately 190 kDa and 70 kDa. However, CDK2- and CTD-kinase activities were immunoprecipitated with R2 antibody in a complex of approximately 105 kDa. Therefore, it seems that R2 protein forms at least two major complexes of 70 kDa and 190 kDa, as well as a minor complex of 105 kDa which is able to phosphorylate CDK2 and CTD. We have recently identified a cyclin H homolog of rice plants (Yamaguchi *et al.*, 2000). Since cyclin H stimulates the CAK activity of Cdk7/p40^{M015}, it is likely that the R2 complex of 105 kDa also contains a potentially active cyclin H (Fig. 3).

R2 phosphorylates rice Cdc2Os1, while two other CDKs from rice, Cdc2Os2 and Cdc2Os3, are not phosphorylated (Yamaguchi *et al.*, 1998). In human, CAK phosphorylates CDC2 which is associated with cyclin B but not CDC2 monomers *in vitro*, whereas CDK2 is phosphorylated without binding of cyclins (Desai *et al.*, 1995; Fisher and Morgan, 1994). Therefore, rice Cdc2Os2 and Cdc2Os3 might require their own cyclin partners before they can serve as substrates for R2. Preliminary results from our laboratories suggest that Cdc2Os3 interacts with one of the two B2-type cyclins (unpublished results), thus Cdc2Os3 may be activated by binding to B2-type cyclin(s), followed by phosphorylation with R2, during the G2-to-M phase (Fig. 3). In contrast, R2 would phosphorylate Cdc2Os1 monomer, and the phosphorylated Cdc2Os1 may interact with particular cyclins to exert its function at each stage of the cell cycle (Fig. 3).

Perspective

Several cell cycle regulators of plants have been identified, thus we can use them as a tool to dissect the molecular processes that regulate the activation or inhibition of cell division. Cell differentiation should be controlled based on a balance with cell

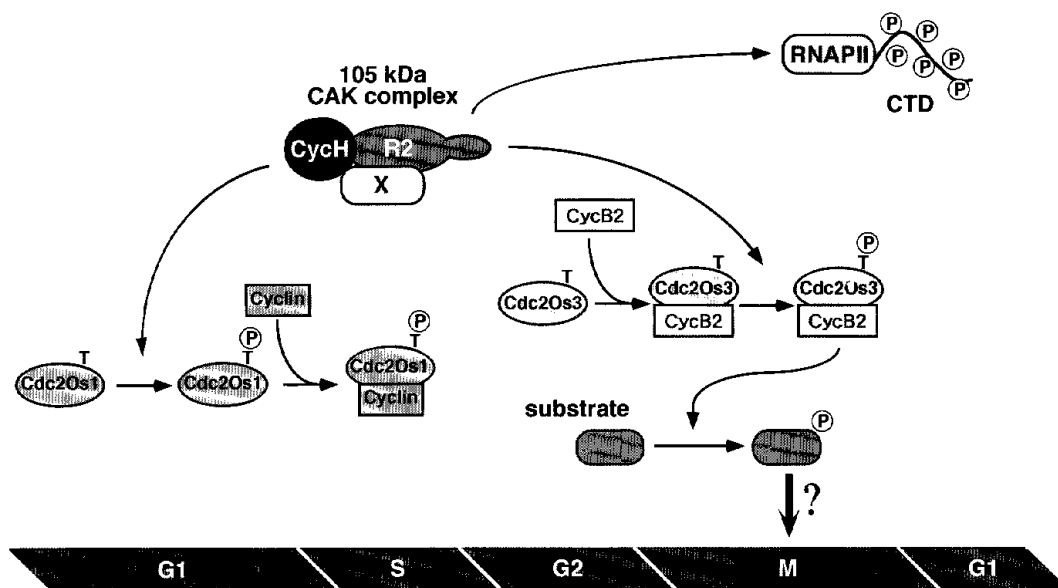


Fig. 3 CDK activation by CAK in rice plant. The rice Cdk7/p40^{MO15} homolog, R2, forms an active kinase complex of 105 kDa with a regulatory subunit, cyclin H (CycH), and another unknown subunit (X). R2 phosphorylates monomer Cdc2Os1, a functional homolog of CDC2/CDC28, but not monomer Cdc2Os3, a CDK that is specifically expressed during the G2-to-M phase. Therefore, a cyclin partner (CycB2) will first bind to Cdc2Os3 which is then phosphorylated by R2 to be fully active in phosphorylation of substrates that regulate mitosis. R2 is also involved in basal transcription by phosphorylating the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II (RNAPII).

division to accomplish proper development of organs. Therefore, it is likely that CDK activity plays a major role in determination of cell fate in meristems. In other words, CDK activity must be tightly regulated by a signaling cascade that perceives environmental conditions and/or phytohormones. Post-translational regulation of cell cycle actors enables plant cells to flexibly control the CDK activity in response to internal or external stimuli. Thus, to identify the molecular mechanisms underlying meristem functions, factors involved in CDK regulation must be characterized biochemically, and the function of such factors should be carefully analyzed by manipulating their activities *in vivo* using transgenic techniques. This approach will facilitate our understanding of any cross talk between signals controlling cell division and cell differentiation, and a key element will be identified with the help of *Arabidopsis* genetics that characterize mutants with defects in meristem activity. Such an effort should highlight the fundamental difference between animals and plants in terms of totipotency of cells.

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