Control of Cell Division and Transcription by Cyclin-dependent Kinase-activating Kinases in Plants

Masaaki Umeda, Akie Shimotohno and Masatoshi Yamaguchi
Institute of Molecular and Cellular Biosciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-0032 Japan

Cyclin-dependent protein kinases (CDKs) play key roles in the progression of the cell cycle in eukaryotes. A CDK-activating kinase (CAK) catalyzes the phosphorylation of CDKs to activate their enzyme activity; thus, it is involved in activation of cell proliferation. In plants, two distinct classes of CAK have been identified; CDKD is functionally related to vertebrate-type CAKs, while CDKF is a plant-specific CAK having unique enzymatic characteristics. Recently, CDKF was shown to phosphorylate and activate CDKDs in Arabidopsis. This led to a proposal that CDKD and CDKF constitute a phosphorylation cascade that mediates environmental or hormonal signals to molecular machineries that control the cell cycle and transcription. In this review, we have summarized the biochemical features of plant CAKs and discussed the manner in which they diverge from animal and yeast orthologs. We have introduced several transgenic studies in which CAK genes were used as a tool to modify the CAK activity and to analyze cell division and differentiation during organ development.

Keywords: CDK-activating kinase — Cell cycle — Cyclin — Cyclin-dependent kinase — Protein phosphorylation — Transcription.

Abbreviations: CAK, CDK-activating kinase; CAKAK, CAK-activating kinase; CDK, cyclin-dependent kinase; CTD, C-terminal domain of the largest subunit of RNA polymerase II; GFP, green fluorescent protein; NER, nucleotide excision repair; NLS, nuclear localization signal; TBP, TATA box-binding protein; T-DNA, transferred DNA.

Introduction

The regulation of cell division in eukaryotes is one of the important mechanisms that underlie several essential processes, including cell fate determination, differentiation, organ development, growth, cell death, carcinogenesis, etc. In comparison with the wealth of information available regarding various components and mechanisms of cell cycle regulation in yeast and animals, our current knowledge on the control of cell division and its role in the regulation of developmental processes in plants is very limited. Recently, the plant cell cycle has become the focus of interest because almost all aspects of post-embryonic development in plants are primarily controlled by cell division in the meristems. Meristems are stem cell tissues that play a role in both self-maintenance and cell production for growth. These two processes are localized in distinct meristem domains having different rates of proliferation.

Studies in yeast, Drosophila and mammals demonstrated that signaling pathways regulating cell cycle progression ultimately converge on the control of activity of cyclin-dependent protein kinases (CDKs). The kinase activity of CDKs is dependent on binding to cyclins. Similarly to vertebrates, plants express several types of CDKs and cyclins (Mironov et al. 1999); thus, different sets of cyclin–CDK pairs might regulate the division of plant cells at each stage of the cell cycle. Based on the primary structure, plant CDKs have been classified into six types, namely, CDKA–CDKF (Joubès et al. 2000, Vandepoele et al. 2002). CDKA contains a conserved PSTAIRE motif, an important domain for cyclin binding, and appears to playa role in both G1 to S and G2 to M-phase progression. In contrast, the expression of CDKB, which has altered PSTAIRE sequences, is restricted from late S to M phases. CDKC and CDKE are related to vertebrate CDK9 and CDK8, respectively. Arabidopsis CDK9 was shown to bind to a cyclin T homolog in a yeast two-hybrid system and immunoprecipitation assays (Barrôco et al. 2003). Wang and Chen (2004) found that CDKE is encoded by HUA ENHANCER3 (HEN3), which is required for the specification of stamen and carpel identities and for the proper termination of stem cells in the floral meristem. However, the precise functions of CDKC and CDKE remain unknown. Recently, a distinct CDK class named CDKG was defined based on the conserved PLTSLRE motif and its homology to the human galactosyltransferase-associated protein kinase p58/GTA, a member of a p34 (Cdc2)-related kinase subfamily (Menges et al. 2005).

The activity of CDKs is also regulated by phosphorylation. A CDK-activating kinase (CAK) phosphorylates a conserved threonine residue on the T-loop region, which blocks the entry of the substrate to the catalytic cleft when the threonine residue is unphosphorylated (for a review, see Nigg 1996, Draetta 1997). Thus, CAK plays an essential role in cell cycle...
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regulation through CDK activation. In this review, we look at the findings from recent studies on plant CAKs. These studies revealed that plants developed a novel CDK phosphorylation cascade mediated by multiple CAKs. We introduce several studies in which the CDK activity was modulated in vivo, facilitating our understanding of cell division and differentiation during organ development.

CAKs in vertebrate and yeast

The catalytic subunit of CAK belongs to the CDK family; it is termed CDK7/p40\textsuperscript{MO15} in vertebrates, and its regulatory subunit is named cyclin H (Fisher and Morgan 1994, Labbé et al. 1994, Mäkelä et al. 1994) (Table 1). CDK7 alone has a low CAK activity, whereas in the presence of cyclin H, CDK7 activity is significantly stimulated. In addition to cyclin H, MAT1, a RING finger protein, also interacts with CDK7 to stabilize the CDK7–cyclin H complex (Devault et al. 1995, Tassan et al. 1995). The other enzyme activity of the vertebrate CAK is to phosphorylate the C-terminal domain (CTD) of the largest subunit of RNA polymerase II. In fact, the trimeric CDK7–cyclin H–MAT1 complex has been identified in the general transcriptional factor TFIIH that is involved in the initiation and elongation of transcription (Serizawa et al. 1995, Shiekhattar et al. 1995). Therefore, vertebrate CAKs have a dual function in cell cycle regulation and basal transcription (Fig. 1).

In fission yeast (\textit{Schizosaccharomyces pombe}), CAK consists of Mcs6/Crk1/Mop1, Mcs2 and Pmh1, which are closely related to CDK7, cyclin H and MAT1, respectively, and phosphorylates both Cdc2 and CTD (Buck et al. 1995, Damagnez et al. 1995) (Table 1). Similarly, in budding yeast (\textit{Saccharomyces cerevisiae}), Kin28p associates with a cyclin H homolog Ccl1p and a MAT1 homolog Rig2p/Tfb3p and phosphorylates CTD as components of TFIIH (Feaver et al. 1994, Cismowski et al. 1995, Svejstrup et al. 1996a, Faye et al. 1997, Feaver et al. 1997) (Table 1). However, this trimeric complex does not exhibit Cdc28p kinase activity. Another protein kinase, designated Cak1p/Civ1p, has been shown to possess CAK activity in vivo (Espinoza et al. 1996, Kaldis et al. 1996, Thuret et al. 1996). Cak1p consists of a single polypeptide that has a very low sequence similarity to other CDKs and CAKs and does not possess CTD kinase activity, indicating that phosphorylation of CDK and CTD is controlled by distinct kinases in budding yeast (Fig. 1).

Table 1 Protein kinases regulating activation of CDK and basal transcription

<table>
<thead>
<tr>
<th>Organism</th>
<th>Catalytic subunit</th>
<th>Regulatory subunit</th>
<th>Assembly subunit</th>
<th>CAK activity</th>
<th>CTD kinase activity</th>
</tr>
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<tbody>
<tr>
<td>Human</td>
<td>CDK7</td>
<td>Cyclin H</td>
<td>MAT1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>p42</td>
<td></td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>\textit{Sch. pombe}</td>
<td>Mcs6/Crk1/Mop1</td>
<td>Mcs2</td>
<td>Pmh1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>\textit{S. cerevisiae}</td>
<td>Kin28p</td>
<td>Ccl1p</td>
<td>Rig2p/Tfb3p</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Cak1p/Civ1p</td>
<td></td>
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<td>–</td>
<td>–</td>
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<tr>
<td>Rice</td>
<td>Os;CDK7;1</td>
<td>Os;CycH;1</td>
<td>?</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Arabidopsis</td>
<td>At;CDK7;1</td>
<td>At;CycH;1</td>
<td>?</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>At;CDK2;1</td>
<td>At;CycH;1</td>
<td>?</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>At;CDK3;3</td>
<td>At;CycH;1</td>
<td>?</td>
<td>Very high</td>
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Fig. 1 Phosphorylation of CDK and CTD by CAKs. In human, CDK7 phosphorylates both CDK and CTD; however, a CAK-activating kinase (CAKAK) remains unknown. Fission yeast Csk1 phosphorylates Mcs6, which has both Cdc2 and CTD kinase activities. Budding yeast Cak1p phosphorylates and activates not only Cdc28p but also the CTD kinase Kin28p. In \textit{Arabidopsis}, At;CDKF;1 phosphorylates and activates not only Cdc28p but also At;CDKD;2 and At;CDKD;3, which preferentially phosphorylate CTD and CDK, respectively.
Plant CDKD is a functional homolog of vertebrate CDK7

Plant CDK7 homologs have been classified into the CDKD group according to the nomenclature proposed by Joubès et al. (2000). The first plant CAK ortholog was identified in rice as a Cdc2-related protein kinase named R2 (Hata et al. 1991), thereafter renamed Os;CDKD;1. A structural difference from vertebrate-type CAKs is that it contains an extended C-terminal region of 92 amino acids (Fig. 2A). Fabian-Marwedel et al. (2002) showed that this extended region contains a nuclear localization signal (NLS). When Os;CDKD;1 was overexpressed in a cak1/civ1-deficient mutant of budding yeast, it partially suppressed the temperature sensitivity. In addition, immunoprecipitates of rice proteins with the anti-Os;CDKD;1 antibody phosphorylated the threonine residue within the T-loop of rice CDKA;1 and the Arabidopsis CTD (Yamaguchi et al. 1998) (Table 1). These data suggest that Os;CDKD;1 is a functional homolog of vertebrate CAKs.

There are three CDKD genes in the Arabidopsis genome, namely At;CDKD;1, At;CDKD;2 and At;CDKD;3 (originally named CAK3At, CAK4At and CAK2At, respectively) (Umeda 2002, Shimotohno et al. 2003). With the exception of At;CDKD;2, both At;CDKD;1 and At;CDKD;3 have the C-terminal extension along with a significant sequence similarity to that of Os;CDKD;1 (Fig. 2A). The green fluorescent protein (GFP) fused to At;CDKDs showed that At;CDKD;1 and At;CDKD;3 displayed almost exclusive nuclear localization, whereas At;CDKD;2 localized in the cytoplasm and nuclei in plant cells (Shimotohno et al. 2004). This supports the idea that the C-terminal region contains the NLS as described earlier. Analysis of their enzyme activities demonstrated that At;CDKD;2 and At;CDKD;3 phosphorylated both human CDK2 and Arabidopsis CTD, whereas At;CDKD;1 lacks these kinase activities in vitro (Shimotohno et al. 2003, Shimotohno et al. 2004) (Table 1). Therefore, At;CDKD;1 may be an inactive CAK variant, or it may have a different substrate specificity. Interestingly, the preference for substrates was different between At;CDKD;2 and At;CDKD;3. The CDK2 kinase activity of At;CDKD;3 was higher than that of At;CDKD;2, whereas At;CDKD;2 had higher CTD kinase activity than At;CDKD;3 (Fig. 1).

In higher plants, cyclin H homologs have been isolated from poplar, rice and Arabidopsis (named Pt;CycH;1, Os;CycH;1 and At;CycH;1, respectively) (Yamaguchi et al. 2000, Shimotohno et al. 2004). In the cyclin box region, plant cyclin H shows approximately 60% similarity to human cyclin H. MAT1 homologs in vertebrates and yeast have two motifs: an N-terminal RING finger domain that plays a crucial role in basal transcription and the CTD phosphorylation process, and a central coiled-coil domain that is associated with XPD and XPB helicases, components of TFIIH complexes (Busso et al. 2000). The C-terminals of MAT1 is also important for association and activity of the vertebrate-type CAK complex (Busso et al. 2000). Arabidopsis and rice genomes contain putative MAT1 homologs (At4g30820 in Arabidopsis). However, their amino acid sequences are conserved only in the coiled-coil domain, and they lack the RING finger motif. To determine whether these genes encode functional MAT1 homologs, further biochemical characterization is essential. CDKDs form multiple CAK complexes with distinct enzyme activities in
plant cells (Yamaguchi et al. 1998, Shimotohno et al. 2004). However, the identity of the complex that contains cyclin H and/or MAT1 homologs is unknown.

Involvement of CDKD in cell cycle regulation and transcription

Transcripts of Os;CDKD;1 and Os;CycH;1 were up-regulated in S phase in partially synchronized rice suspension cells, and the CTD kinase activity of Os;CDKD;1 was also elevated in S phase (Sauter 1997, Yamaguchi et al. 2000, Fabian-Marwedel et al. 2002). In the intercalary meristem of deepwater rice internodes, the transcript level and the kinase activity of Os;CDKD;1 significantly increased by submergence or by gibberellin treatment to accelerate the cell division. This up-regulation showed a good correlation with the increase of S-phase cells at early time points after submergence (Sauter et al. 1995, Sauter 1997). Therefore, Os;CDKD;1 may control S-phase entry and/or progression through DNA replication and activation of downstream CDKs. To support this idea, the rice suspension cells overexpressing Os;CDKD;1 accelerated S-phase progression and increased the ratio of G2-phase cells (Fabian-Marwedel et al. 2002). In contrast, microarray analysis of Arabidopsis cell cycle regulators showed that At;CDKD;2 and At;CDKD;3 are constantly expressed throughout the cell cycle. Rather, At;CDKD;3 was up-regulated after sucrose removal and resupply in suspension cells (Menges et al. 2005). This indicates that At;CDKD;3 may play a role in the activation of CDK activity during cell cycle re-entry.

The bacterial virulence protein VirD2 plays an important role in the nuclear import and chromosomal integration of Agrobacterium-transferred DNA (T-DNA) in eukaryotic host cells. Recently, Bakó et al. (2003) found that VirD2 interacted with and was phosphorylated by an alfalfa CDKD, Ms;CDKD;1 (originally designated as CAK2Ms), in vitro and in vivo. They also found that VirD2 was tightly associated with a TATA box-binding protein (TBP) in the nuclei of alfalfa cells. The TBP protein is a key regulator in transcription/repair systems via its interaction with the basic transcription machinery, including RNA polymerase II. Similarly to vertebrate-type CAKs, Ms;CDKD;1 interacted with and phosphorylated the CTD, which could recruit TBP (Bakó et al. 2003). VirD2 interactions with TBP and CDKD suggest that T-DNA integration may be mediated by widely conserved nuclear factors in eukaryotes. Mammalian TFIIH is involved in nucleotide excision repair (NER) as well as in the initiation and elongation of transcription (Svejstrup et al. 1996b). Therefore, it is likely that nuclear VirD2-binding factors provide a link between T-DNA integration and transcription-coupled repair through association with components of TFIIH. It will be interesting to determine whether phosphorylation of VirD2 by CDKD would regulate their interactions and affect nuclear import and integration of the T-DNA in Agrobacterium-transformed cells.

Arabidopsis CDKF is a distinct type of CAK

At;CDKF;1 (originally designated as CAK1At) has been isolated as a suppressor of the CAK mutation in budding yeast (Umeda et al. 1998). The amino acid sequence is related to those of vertebrate-type CAKs, but similarities are restricted to the conserved kinase domains. A unique feature of At;CDKF;1 among the other CAKs is that it carries an unusual insertion of 111 amino acids between its kinase active site and the phospho-regulatory site (Fig. 2B). Using database searches, homologs of Arabidopsis CDKF;1 can be identified only in plant species, namely Euphorbia, rice and soybean, but not in other kingdoms. All the four plant species contain the unique insertion with significant amino acid similarity (Fig. 2B), suggesting that this region may be involved in the control of interactions of CDKF with specific regulatory proteins or substrates acting in plant-specific signaling pathways.

Immunoprecipitates of Arabidopsis protein extract with the anti-At;CDKF;1 antibody phosphorylated human CDK2 at the threonine residue within the T-loop and activated its histone H1 kinase activity. However, At;CDKF;1 did not phosphorylate the Arabidopsis CTD in vitro and was unable to interact with At;CycH;1 (Umeda et al. 1998, Shimotohno et al. 2004) (Table 1). These results indicate that At;CDKF;1 has a CDK-activating kinase activity, but is distinct from vertebrate-type CAKs, including plant CDKDs. At;CDKF;1 occurs in a 130 kDa active protein complex in suspension cells (Shimotohno et al. 2004); thus, it is likely that At;CDKF;1 is associated in vivo with other regulatory protein(s) that might control its activity in response to external or internal stimuli.

CDKF is the plant-specific CAK-activating kinase

Vertebrate CDK7 and fission yeast Mcs6 are also phosphorylated at serine and threonine residues within their T-loops, similarly to other CDKs. In fission yeast, Csk1, which has been identified as the second kinase with CAK activity, was found to phosphorylate Mcs6 on the T-loop activation site (S165) and activate the Mcs6–Mcs2 complex in vivo (Hermand et al. 1998, Lee et al. 1999, Hermand et al. 2001) (Fig. 1). Thus, Csk1 was defined as CAK-activating kinase (CAKAK), an upstream activating kinase of Mcs6. In budding yeast, Kimmelman et al. (1999) demonstrated that Cak1p phosphorylated the T-loop of Kin28p and thereby stimulated its CTD kinase activity (Fig. 1). This suggests that despite their low sequence similarity, budding yeast Cak1p and fission yeast Csk1 perform similar functions by phosphorylation of Kin28p and Mcs6, respectively. In fact, detailed phylogenetic analyses revealed that Cak1p and Csk1 were included in the same family with significant bootstrap support (Liu and Kipreos 2002).

When Arabidopsis CAK cDNAs were introduced into a fission yeast strain which carries a disrupted csk1Δ gene, only transformants expressing At;CDKF;1, but not any At;CDKD, could suppress its temperature sensitivity. Moreover, an in vitro kinase assay demonstrated that At;CDKF;1 phosphorylated the conserved serine and threonine residues within the T-loops of...
At;CDKD;2 and At;CDKD;3, but not At;CDKD;1. In Arabidopsis root protoplasts, the CTD kinase activity of At;CDKD;2 was elevated depending on its T-loop phosphorylation by At;CDKF;1 (Shimotohno et al. 2004). These results suggest that At;CDKD;1 is a CAKAK that modulates the activity of At;CDKD;2 and At;CDKD;3, thereby controlling CDK activities and basal transcription in Arabidopsis (Fig. 1). Recently, Liu et al. (2004) identified a novel monomeric CAK p42 in human cells, which shows sequence homology to both Cak1p and CDK7. Although the enzyme activity in vitro was rather weak, it was responsible for CDK2-activating kinase activity in vivo (Table 1). However, p42 had no CDK7-activating kinase activity; thus, CAKAK in vertebrates remains to be identified. As described earlier, CDKF-related genes have been identified only in the plant kingdom, indicating that the phosphorylation cascade mediated by CDKD and CDKF may receive environmental and/or hormonal signals to achieve proper development of plant tissues.

Studies on plant cell division and differentiation by modulating the CDK activity

CAK is engaged in activation of almost all CDKs. Thus, CAK genes can be a tool to modulate the overall CDK activity in tissues. When the sense or antisense gene of At;CDKD;1 was expressed in Arabidopsis in an inducible manner, the kinase activity of At;CDKA;1 was reduced, and root growth was inhibited. Prior to the cessation of cell division, columna and cortical initial cells were differentiated into daughter cells in the root meristem (Umeda et al. 2000). This indicates that the indeterminate state of the initial cells might be maintained by CDK activities independent of cell division. Another example is the overexpression of Os;CDKD;1 in tobacco leaf explants (Yamaguchi et al. 2003). In root-inducing media, root regeneration was markedly inhibited in transgenic leaf sections. Moreover, Os;CDKD;1-expressing explants produced calli in the presence of high concentration of auxin. This result indicates that endogenous CDK activities were elevated by Os;CDKD;1 overexpression, and root regeneration was converted to disorganized cellular proliferation resulting in production of calli. These two instances suggest that cell differentiation is governed within a particular range of CDK activity.

Similar observations have been described in transgenic plants overexpressing cyclin genes. Weingartner et al. (2003) produced transgenic tobacco plants that ectopically expressed an alfalfa cyclin B2 gene. Leaf disk assays showed that root regeneration from transgenic sections was blocked in a medium containing a high auxin to cytokinin ratio. In plants, cyclin D3 overexpression in Arabidopsis inhibited the differentiation of leaves with small polygon-shaped pavement cells and retarded the formation of lignified secondary xylem elements (Dewitte et al. 2003). These observations led us to the proposal that molecular mechanisms suppressing the CDK activity under a threshold level play an essential role in differentiation programs during organ development.

Conclusion and perspectives

In plants, CDKD is functionally related to vertebrate-type CAKs. CDKD is associated with and activated by a regulatory partner cyclin H and has both CDK and CTD kinase activities. Interestingly, in Arabidopsis, multiple CDKDs display distinct substrate preferences, suggesting that they may play different roles in the cell cycle, transcription, DNA repair and chromosomal integration of Agrobacterium T-DNA. CDKF, which does not interact with cyclin H, has a high CDK-activating kinase activity, but does not possess CTD kinase activity. CDKF is also involved in phosphorylation and activation of CDKDs, indicating that there exists a CDK and/or CTD phosphorylation cascade mediated by multiple CAKs. CDKF-related proteins have been identified only in plant species, indicating that CDKF is the first CAKAK that has been characterized in multicellular organisms. It is intriguing to identify hormonal and developmental signals that control the CDKF activity in plant cells. We described that cell cycle genes, such as CAK and cyclin genes, can be used to modify the CDK activity in vivo. Further studies on transgenic plants in which CDK activities are modulated in a tissue-specific manner will help us to determine the process by which cell division and differentiation are coordinately regulated in meristems to accomplish continuous development during the life cycle.

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