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REVIEW

Cyclin dependent kinases and their role in regulation of plant cell cycle

J.G. TANK and V.S. THAKER*

Centre for Advanced Studies in Plant Biotechnology and Genetic Engineering, Department of Biosciences, Saurashtra University, Rajkot-360005, India

Abstract

Plants have capability to optimize its architecture by using CDK pathways. It involves diverse types of cyclin dependent kinase enzymes (CDKs). CDKs are classified in to eight classes (CDKA to CDKG and CKL) based on the recognized cyclin-binding domains. These enzymes require specific cyclin proteins to get activated. They form complex with cyclin subunits and phosphorylate key target proteins. Phosphorylation of these target proteins is essential to drive cell cycle further from one phase to another phase. During cell division, the activity of cyclin dependent kinase is controlled by CDK interactor/inhibitor of CDKs (ICK) and Kip-related proteins (KRPs). They bind with specific CDK/cyclin complex and help in controlling CDKs activity. Since cell cycle can be progressed further only by synthesis and destruction of cyclins, they are quickly degraded using ubiquitination-proteasome pathway. Ubiquitylation reaction is followed by DNA duplication and cell division process. These two processes are regulated by two complexes known as Skp1/cullin/F-box (SCF)-related complex and the anaphase-promoting complex/cyclosome (APC/C). SCF allows cell to enter from G1 to S phase and APC/C allows cell to enter from G2 to M phase. When all these above processes of cell division are going on, genes of cyclin dependent kinases gets activated one by one simultaneously and help in regulation of CDK pathways. How cell cycle is regulated by CDKs is discussed.

Additional key words: anaphase-promoting complex, cell division, cullin protein, Skp1/cullin/F-box (SCF), ubiquitination-proteasome pathway.

Introduction

The development of plant organs is directly dependent on the frequency of cell division, parameters of the cell cycle and the number and size of the cells. These processes are controlled by molecular machinery that regulates the cell cycle progression in coordination with nutritional, hormonal, developmental and environmental signals. Molecular machinery involves orderly progression of cells through the various phases of the cell cycle and their appropriate responses to extracellular clues. These are governed by multiple regulatory mechanisms such as reversible protein phosphorylation, interaction of proteins and specific protein degradation (Inze and De Veylder 2006). Protein phosphorylation is one of the widely known major mechanisms that control progression of cell cycle. It influences cell cycle by altering the activity of proteins, sub-cellular localization of proteins, degradation of target proteins and dynamic changes in protein complexes. It is carried out by enzymes known as kinases. Among the diverse kinases, family of cyclin-dependent protein kinases (CDKs) is significant for cell division control. They form complex with cyclin subunits and drive the cell cycle further by phosphorylating key target proteins which are required for cells to progress from one phase to the next.

The first complete list of 46 putative plant CDK genes were published by Joubes *et al.* (2000) from 23 species by considering sequence of amino acids of five classes of CDK (CDKA to CDKE), depending on the similarities

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Abbreviations: APC/C - anaphase-promoting complex/cyclosome; CAKs - CDK-activating kinases; CDK - cyclin dependent kinases; CKL - CDK-like; CSN - COP9-signalosome; CTD - C-terminal domain; CUL - cullin; CYCT - cyclin T; DUBs - deubiquitylating enzymes; E1 - ubiquitin-activating enzyme; E2 - ubiquitin-conjugating enzyme; ECS - elonginC-CUL2-SOCS-box; HECT - homologous to the E6-AP C-terminus; HEN3 - HUA enhancer 3; HUA - flower in Chinese; ICK - interactor/inhibitor of CDKs; KRPs - Kip-related proteins; PPB - preprophase band; RBR - retinoblastoma-related protein; SCF - Skp1/cullin/F-box; UPS - ubiquitin-dependent proteolysis.

* Corresponding author; fax: (+91) 281 258 6419, e-mail: cpbge@yahoo.co.in

and characteristic motifs identified. This list was further extended into 152 CDK genes from 41 plant species. The putative cyclin-binding domains served as criteria for classification of CDKs. So, the CDK proteins were categorized into eight classes: CDKA to CDKG and cyclin dependent kinases like (CKL; Fig. 1). CDKs such as A-type with PSTAIRE motif, B-type with PPTALRE or PPTTLRE motif; C-type with PITAIRE motif, D, E and F-type CDKs regulating CDK activators, with SPTAIRE motif; G-type CDKs with PLTSLRE motif and CKL-type with (V,I,L) (K,R) FMAREI motif, where residues change in motif depending on the type of CKL range from CKL1 to CKL15.

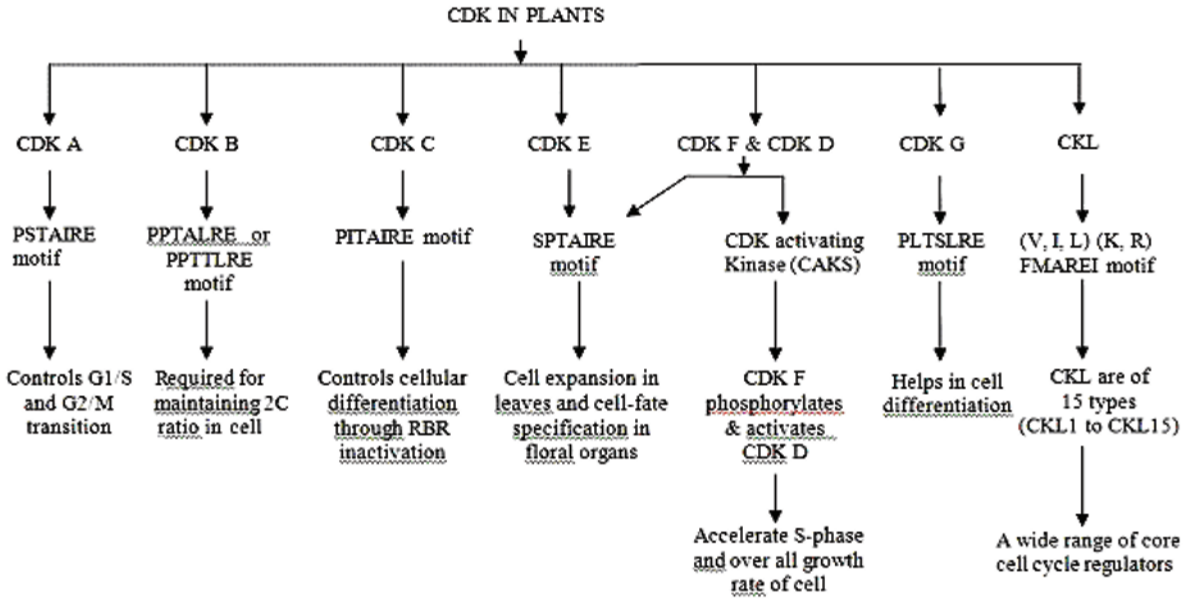

Fig. 1. Types of CDKs in plants.

Types of CDKs in plants

CDKA: Class CDKA is the largest class among the plant CDK family with 48 members. It has a conserved PSTAIRE motif, which is responsible for binding to cyclins and a specific feature which separate it from other CDKs. The comprehensive data collected from previous studies shows number of species with two types of CDKA kinases positioned in separate subgroups. In monocotyledonous grasses such as rice, wheat and maize a characteristic branch is formed that separates two subgroups. Similarly, species from families of dicotyledons such as *Solanaceae, Fabaceae* and *Umbelliferae (Apiaceae)* also exhibit closely related CDKA kinases. However, in *Arabidopsis thaliana* only one A-type of *CDK* gene called *CDC2A* or *CDKA;1* has been found till today (Table l).

Immunofluorescence studies have shown that A-type CDKs are found largely in the interphase and early prophase nucleus and to a lesser extent in the cytoplasm in maize, tobacco and *Arabidopsis* (Mews *et al.* 2000, Dissmeyer *et al.* 2007). During mitosis, A-type CDKs have been found in association with a number of cytoskeletal structures, such as the preprophase band (PPB), spindle, and phragmoplast (Criqui *et al.* 2001). The mitotic activity of *CDKA;1* and the localization of *CDKA;1* with mitotic microtubuli structures, such as the preprophase band and metaphase spindle, indicates a mitotic role of A-type CDKs (Boruc *et al.* 2010). They transiently interact with chromosomes at the metaphase-anaphase transition (Stals *et al.* 1997). Bögre *et al.* 1997 have observed that comparable amounts of A-type CDKs are present in the cytoplasmic and nuclear fractions of alfalfa cells during S phase. It expresses throughout the cell cycle (Hirayama *et al.* 1991). Overexpression of a dominant negative type of CDKA in plants revealed that CDKA is involved in controlling both G1/S and G2/M transitions (Hemerly *et al.* 1995). Two very homologues *CDK A* genes of alfafa *Medsa*; *CDKA;1;1* and *Medsa*; *CDKA;2;1* displayed a diverse ability of complement yeast cdc28 mutants whose cell cycle was blocked at G1-S and G2-M phases suggesting different role of CDKA genes in cell cycle regulation (Hirt *et al.* 1993). Overexpression of a dominant negative form of the *Arabidopsis CDKA;1* gene in tobacco plants, resulted in an overall reduction of cell division rate, thus yielding smaller plants. However, the G1/G2 ratio remains unaltered, demonstrating that *CDKA;1* is essential at both G1/S and G2/M transition of the cell cycle (Hemerly *et al.* 1995). Genes of CDKA identified from different plant species are listed in Table 1.

CDKB: Class CDKB is the second largest group of plant CDKs, these kinases are plant specific and are involved in divergent cellular functions (De Veylder *et al.* 2007). It is known as a plant-specific CDK, because it has altered PSTAIRE motif, and its expression is under strict cell cycle control. The discovery of the non-PSTAIRE Cdc2 kinases in *Arabidopsis* (cdc2bAt: PPTALRE; Segers *et al.* 1996) and in alfalfa (Cdc2MsD: PPTALRE; Cdc2MsF: PPTTLRE; Magyar *et al.* 1997) revealed that it is a new category of CDKs. Joubes *et al.* (2000) proposed that PPTALRE Cdc2 kinases should be placed in class CDKB1 and PPTTLRE Cdc2 kinases in class CDKB2. Hence, the present class of CDKB is definitely divided into two major groups: CDKB1 with the PPTALRE motif which is expressed from S to M phase, and CDKB2 with the P(S/P) TTLRE motif which is expressed in a more restricted period from G2 to M phase (Mészáros *et al.* 2000). For dicot species such as *Arabidopsis*, tobacco and alfalfa, both class CDKB1 and class CDKB2 are represented by two members. However among monocot grass species, class CDKB2 is not recognized till today. Though PPTALRE motif belongs to class CDKB1, it is visible in the CDKB tree that a small group of monocot CDKB1 genes anomalously form a conspicuous cluster, which is attracted to the CDKB2 clade. Recent studies showed that overexpression of a dominant negative type of *Arabidopsis* CDKB1;1 delayed the G2-to-M transition in tobacco cells (Porceddu *et al.* 2001), suggesting that at least CDKB1 is involved in mitotic entry. Immunofluorescence studies have also shown that localization pattern of CDKB1;1 in tobacco BY2 cells was nuclear and cytoplasmic in interphase cells and diffused to the cytoplasm upon nuclear envelope breakdown. Later, it weakly associated with the phragmoplast (Boruc *et al.* 2010). CDKB2;1 is expressed from early G2 to M phase, and CYCD4;1 is expressed throughout the cell cycle. Determination of the mitotic index, flow-cytometric analysis, and reverse transcription-PCR experiments has also concluded that *Medsa; CDKB2;1* gene has promoter activity that is characteristic for proliferating cells with G2/M cell cycle phase specificity (Zhiponova *et al.* 2006). Both of these CDKs form an active kinase complex and control G2/M phase transition and mitotic events.

The recent genome-wide transcript profiling of the core *Arabidopsis* cell cycle *via Affymetrix* microarrays done by Menges *et al.* (2005) refined the previous data of the expression pattern of the *CDKB* genes (Table 1) which showed an early G2 peak for the *CDKB1;1/2* genes. Also, alfalfa cells synchronized with aphidicolin showed the gene *Medsa;CDKB1;1* (*cdc2MsD*) expressed earlier in the G2 phase than the gene *Medsa,CDKB2;1* (*cdc2MsF*) in the late G2/M phase. In these cells, the *Medsa;CDKB2;1* transcripts were more abundant than those synthesized from the *Medsa;CDKB1;1* genes (Magyar *et al.* 1997). Thus, *CDKB* genes transcribe actively in S phase (Menges *et al.* 2005). Localization of CDKB at the preprophase band, metaphase plate and its mitotic activity suggests the role of B-type CDKs in the control of mitosis (Lee *et al.* 2003). In rice (*Oryza sativa*), the mitotic CyclinB2;2 was shown to activate CDKB2;1, and both proteins co-localize on chromosomes at metaphase, suggesting that this complex may be involved in the control of mitosis (Lee *et al.* 2003). Role of CDKB in integration of developmental pathways is also clearly demonstrated. *Arabidopsis* plants overexpressing a dominant negative *CDKB1;1* (*CDKB1;1 N161*) display cells with a higher 4C/2C ratio in specific tissues. This was correlated to dramatic consequences on stomata development (Boudolf *et al.* 2004a). In the leaves of *CDKB1;1N161* plants, cells prematurely exit mitotic cycle and enter endo-reduplication cycles associated with leaf development (Boudolf *et al.* 2004b). Thus, *CDKB1;1* is implicated in both the control of cell cycle progression and integration of developmental pathways. Genes of CDK B identified from different plant species are listed in Table 1.

CDKC, CDKD, CDKE, CDKF, CDKG and CKL: Class CDKC kinases carrying the PITAIRE cyclin-binding motifs are the closest homologues of the metazoan CDK9 proteins. It has no clear role in cell cycle control. It contains PITAIRE or SPTAIRE hallmarks that interacts with CYCT, and plays a presumed role in transcription elongation by phosphorylating the CTD of RNA polymerase II (Fulop *et al.* 2005). Phylogenetic tree analysis had showed that this group of CDKs is closely related to the CKLs, which was demonstrated by Menges *et al.* (2005) for *Arabidopsis*. Novel classes of *CKL* genes were discovered by *Affymetrix* microarray analysis by Menges *et al.* (2005). In *Arabidopsis*, 15 CKL proteins formed a cluster well separated from the class CDKC kinases. Genes of CDKC and CKL identified from different plant species are listed in Table 1.

CDKE contains a SPTAIRE motif which was first detected in alfalfa (Magyar *et al.* 1997, Wang and Chen 2004). CDKE is a homologue of mammalian CDK8. It interacts with cyclin C and exerts a negative effect on transcription as a component of the RNA polymerase II holoenzyme (Maldonado *et al.* 1996, Rickert *et al.* 1996). CDKE phosphorylates CTD of RNA polymerase II produced in dividing tissues (Wang and Chen 2004). Classes CDKE and CDKG are less well separated, but they differ in putative cyclin binding motifs. CDKE acts in cell expansion in leaves and cell-fate specification in floral organs (Inze and De Veylder 2006). The CDKG kinases were identified in *Arabidopsis* as proteins containing the PLTSLRE motif. They have role in cell differentiation process (Menges *et al.* 2005). Genes of CDKE identified from different plant species are listed in Table 1.

Classes CDKD and CDKF are members of a kinase network that regulates CDK activity *via* a phospho-rylation cascade. The CDK-activating kinases (CAKs) are CDKD activated by CDKF kinases. Phosphorylation of Thr160 (or the equivalent residue) of CDKs induces a conformational change allowing proper recognition of substrates and is performed by CDK-activating kinases

(CAKs). Four CAK-encoding genes were identified from *Arabidopsis* and were divided into two functional classes (CDKD and CDKF; Umeda *et al.* 2005). The functional diversity between the two CAK classes can be shown by their substrate specificity and cyclin dependence. CAKs play an important role in determining the growth rate and the differentiation status of cells by controlling the overall level of CDK activity. Genes of CDK D and F are listed in Table 1.

Table 1. Sequence details for CDK genes

CDK	Species	Gene name	Accession	Protein ID
CDKA	*Allium cepa*	*Allce;CDKA1;1*	AB006033	BAA21673
	Antirrhinum majus	*Antma;CDKA1;1*	X97637	CAA66233
	Antirrhinum majus	*Antma;CDKA2;1*	X97638	CAA66234
	Arabidopsis thaliana	*Arath;CDKA1;1*	M59198	AAA32831
	Beta vulgaris	*Betvu;CDKA1;1*	Z71702	CAA96384
	Brassica napus	*Brana;CDKA1;1*	U18365	AAA92823
	Camellia sinensis	*Camsi;CDKA1;1*	AB247281	BAE80323
	Chenopodium rubrum	*Cheru;CDKA1;1*	Y10160	CAA71242
	Coffea arabica	*Cofar;CDKA1;1*	AJ496622	CAD43177
	Daucus carota	*Dauca;CDKA1;1*	AJ505322	CAD43850
	Glycine max	*Glyma;CDKA1;1*	M93139	-NA-
	Glycine max	*Glyma;CDKA1;2*	M93140	-NA-
	Helianthus annuus	*Helan;CDKA1;1*	AF321361	AAL37195
	Helianthus tuberosus	*Heltu;CDKA2;1*	AY063462	AAL47481
	Juglans nigra × Juglans regia	*Jugni;CDKA1;1*	AJ439598	CAD29319
	Lycopersicon esculentum	*Lyces;CDKA1;1*	Y17225	CAA76700
	Lycopersicon esculentum	*Lyces;CDKA2;1*	Y17226	CAA76701
	Medicago sativa	*Medsa;CDKA1;1*	M58365	AAB41817
	Medicago sativa	*Medsa;CDKA2;1*	X70707	CAA50038
	Nicotiana tabacum	*Nicta;CDKA1;1*	L77082	AAB02567
	Nicotiana tabacum	*Nicta;CDKA1;2*	L77083	AAB02568
	Nicotiana tabacum	*Nicta;CDKA1;3*	D50738	BAA09369
	Nicotiana tabacum	*Nicta;CDKA1;4*	AF289467	AAG01534
	Oryza sativa	*Orysa;CDKA2;1*	X60374	CAA42922
	Oryza sativa	*Orysa;CDKA1;1*	X60375	CAA42923
	Oryza sativa	*Orysa; CDKA1;2*	AC113930	AAN62789
	Petroselinum crispum	*Petcr;CDKA1;1*	L34206	AAC41680
	Petunia hybrida	*Pethy;CDKA1;1*	Y13646	CAA73997
	Phaseolus vulgaris	*Phavu;CDKA1;1*	AF126737	AAD30494
	Physcomitrella patens	*Phypa;CDKA1;1*	AJ515321	CAD56245
	Picea abies	*Picab;CDKA1;1*	X77680	CAA54746
	Pinus contorta	*Pinco;CDA1;1*	X80845	CAA56815
	Pisum sativum	*Pissa;CDKA1;1*	X53035	CAA37207
	Pisum sativum	*Pissa;CDKA2;1*	AB008187	BAA33152
	Populus tremula × Populus tremuloides	*Poptr;CDKA1;1*	AF194820	AAK16652
	Scutellaria baicalensis	*Scuba;CDKA1;1*	AB205131	BAE06268
	Scutellaria baicalensis	*Scuba;CDKA2;1*	AB205132	BAE06269
	Sesbania rostrata	*Sesro;CDKA1;1*	Z75661	CAA99991
	Solanum tuberosum	*Soltu;CDKA1;1*	U53510	AAA98856
	Triticum aestivum	*Triae;CDKA1;1*	U23409	AAD10483
	Triticum aestivum	*Triae;CDKA2;1*	U23410	AAD10484
	Vigna acunitifolia	*Vigac;CDKA1;1*	M99497	AAA34241
	Vigna radiata	*Vigra;CDKA1;1*	AF129886	AAD30506
	Vigna unguiculata	*Vigun;CDKA1;1*	X89400	CAA61581
	Zea mays	*Zeama;CDKA1;1*	M60526	AAA33479
	Zea mays	*Zeama;CDKA2;1*	BT018184	-NA-
	Zea mays	*Zeama;CDKA1;2*	BT016935	-NA-
	Zea mays	*Zeama;CDKA2;2*	AY104247	-NA-
CDKB	*Antirrhinum majus*	*Antma;CDKB1;1*	X97639	CAA66235
	Antirrhinum majus	*Antma;CDKB2;1*	X97640	CAA66236
	Arabidopsis thaliana	*Arath;CDKB1;1*	D10851	BAA01624
	Arabidopsis thaliana	*Arath;CDKB1;2*	NM001036430	NP001031507

	Arabidopsis thaliana	*Arath;CDKB2;1*	AC015450	AAG51960
	Arabidopsis thaliana	*Arath;CDKB2;2*	NM 101946	NP 173517
	Camellia sinensis	*Camsi;CDKB1;1*	AB247279	BAE80321
	Chenopodium rubrum	*Cheru;CDKB1;1*	AJ278885	CAC17703
	Dunaliella terciolata	*Dunte;CDKB2;1*	AF038570	AAD08721
	Glycine max	*Glyma;CDKB2;1*	AY439096	AAS13369
	Helianthus tuberosus	*Heltu;CDKB2;1*	AY063463	AAL47482
	Lycopersicon esculentum	*Lyces;CDKB1;1*	AJ297916	CAC15503
	Lycopersicon esculentum	*Lyces;CDKB2;1*	AJ297917	CAC15504
	Medicago sativa	*Medsa;CDKB1;1*	X97315	CAA65980
	Medicago sativa	*Medsa;CDKB2;1*	X97317	CAA65982
	Medicago sativa	*Medsa;CDKB2;2*	DQ136188	AAZ30705
	Mesembryanthemum crystallinum	*Mescr;CDKB2;1*	AB015182	BAA28778
	Nicotiana tabacum	*Nicta;CDKB1;1*	AF289465	AAG01532
	Nicotiana tabacum	*Nicta;CDKB1;2*	AF289466	AAG01533
	Oryza sativa	*Orysa;CDKB1;1*	AP003349	BAD82176
	Oryza sativa	*Orysa;CDKB1;2*	D64036	BAA19553
	Populus tremula × *Populus tremuloides*	*Poptr;CDKB2;1*	AY307372	AAP73784
	Scutellaria baicalensis	*Scuba;CDKB1;1*	AB205133	BAE06270
	Sorghum bicolor	*Sorbi;CDKB1;1*	AY144442	-NA-
	Triticum aestivum	*Triae;CDKB1;1*	BT009641	-NA-
	Triticum aestivum	*Triae;CDKB1;2*	BT009182	-NA-
	Zea mays	*Zeama;CDKB1;1*	AY106440	-NA-
	Zea mays	*Zeama;CDKB1;2*	AY106029	-NA-
CDKC	*Arabidopsis thaliana*	*Arath;CDKC;1*	AL360334	CAB96683
	Arabidopsis thaliana	*Arath;CDKC;2*	NM 125895	NP 201301
	Brassica rapa	*Brana;CDKC;1*	AC155344	-NA-
	Brassica rapa	*Brana;CDKC;2*	AC166741	-NA-
	Lycopersicon esculentum	*Lyces;CDKC;1*	AJ294903	CAC51391
	Lycopersicon esculentum	*Lyces;CDKC;2*	BT014075	-NA-
	Medicago sativa	*Medsa;CDKC;1*	X97314	CAA65979
	Oryza sativa	*Orysa;CDKC;2*	AP004326	BAD88154
	Oryza sativa	*Orysa;CDKC;1*	AC105773	AAT47442
	Pisum sativum	*Pissa;CDKC;1*	X56554	CAA39904
	Physcomitrella patens	*Phypa;CDKC;1*	AJ428950	CAD21952
	Zea mays	*Zeama;CDKC;1*	AY107067	-NA-
CDKD	*Arabidopsis thaliana*	*Arath;CDKD1;1*	NM 106028	NP 177510
	Arabidopsis thaliana	*Arath;CDKD1;2*	NM 105345	NP 176847
	Arabidopsis thaliana	*Arath;CDKD1;3*	NM 101666	NP 173244
	Lycopersicon esculentum	*Lyces;CDKD;1*	BT013748	-NA-
	Medicago sativa	*Medsa;CDKD;1*	AF302013	-NA-
	Oryza sativa	*Orysa;CDKD1;1*	X58194	CAA41172
CDKE	*Arabidopsis thaliana*	*Arath;CDKE;1*	AB005234	BAB10454
	Medicago sativa	*Medsa;CDKE;1*	X97316	CAA65981
	Mesembryanthemum crystallinum	*Mescr;CDKE;1*	AB015181	BAA28777
	Oryza sativa	*Zeama;CDKE;1*	AC018727	AAG46164
	Zea mays	*Orysa;CDKE;1*	BT018448	-NA-
CDKF	*Arabidopsis thaliana*	*Arath;CDKF;1*	AB009399	BAA28775
	Euphorbia esula	*Eupes;CDKF;1*	AF230740	AAF34804
	Oryza sativa	*Orysa;CDKF;1*	AP004784	BAD61885
	Glycine max	*Glyma;CDKF;1*	AY439095	AAS13368
CDKG	*Arabidopsis thaliana*	*Arath;CDKG;1*	NM 125732	NP 201142
	Arabidopsis thaliana	*Arath;CDKG;2*	BT000694	AAL32755
	Oryza sativa	*Orysa;CDKG;1*	XM 466592	XP 466592
	Oryza sativa	*Orysa;CDKG;2*	XM 472963	XP 472963
	Zea mays	*Zeama;CDKG;1*	AY112336	-NA-
	Zea mays	*Zeama;CDKG;2*	BT018272	-NA-
	Arabidopsis thaliana	*Arath;CDKG;1*	NM 125732	NP 201142
	Arabidopsis thaliana	*Arath;CDKG;2*	BT000694	AAL32755
	Oryza sativa	*Orysa;CDKG;1*	XM 466592	XP 466592
	Oryza sativa	*Orysa;CDKG;2*	XM 472963	XP 472963
	Zea mays	*Zeama;CDKG;1*	AY112336	-NA-

	Zea mays	*Zeama;CDKG;2*	BT018272	-NA-
CKL	*Arabidopsis thaliana*	*Arath;CDKL;1*	NM 123304	NP 198758
	Arabidopsis thaliana	*Arath;CDKL;2*	NM 106093	NP 177573
	Arabidopsis thaliana	*Arath;CDKL;3*	NM 101725	NP 173302
	Arabidopsis thaliana	*Arath;CDKL;4*	NM 118423	NP 194025
	Arabidopsis thaliana	*Arath;CDKL;5*	NM 001036933	NP 001032010
	Arabidopsis thaliana	*Arath;CDKL;6*	NM 001035873	NP 001030950
	Arabidopsis thaliana	*Arath;CDKL;7*	NM 124464	NP 199899
	Arabidopsis thaliana	*Arath;CDKL;8*	NM 111377	NP 187156
	Arabidopsis thaliana	*Arath;CDKL;9*	NM 104338	NP 175862
	Arabidopsis thaliana	*Arath;CDKL;10*	NM 104567	NP 176083
	Arabidopsis thaliana	*Arath;CDKL;11*	NM 100832	NP 172431
	Arabidopsis thaliana	*Arath;CDKL;12*	NM 202395	NP 974124
	Arabidopsis thaliana	*Arath;CDKL;13*	NM 117069	NP 192739
	Arabidopsis thaliana	*Arath;CDKL;14*	NM 103	NP 174637
	Arabidopsis thaliana	*Arath;CDKL;15*	NM 104184	NP 175713
	Beta vulgaris	*Betvu;CDKL;1*	Z71703	CAA96385
	Beta vulgaris	*Betvu;CDKL;2*	AJ277243	CAB89665
	Lotus japonicus	*Lotja;CDKL;1*	AP004904	-NA-
	Medicago truncatula	*Medtr;CDKL;1*	AC147364	-NA-
	Medicago truncatula	*Medtr;CDKL;2*	AC146747	ABE89393
	Medicago truncatula	*Medtr;CDKL;3*	AC130801	ABE83648
	Medicago truncatula	*Medtr;CDKL;4*	AC149804	ABE79143
	Medicago truncatula	*Medtr;CDKL;5*	AC141113	ABE94624
	Medicago truncatula	*Medtr;CDKL;6*	AC148397	ABE81196
	Oryza sativa	*Orysa;CDKL;1*	AK101089	-NA-
	Oryza sativa	*Orysa;CDKL;2*	NM 193805	NP 918694
	Oryza sativa	*Orysa;CDKL;3*	AK064909	-NA-
	Oryza sativa	*Orysa;CDKL;4*	NM 188289	NP 913178
	Oryza sativa	*Orysa;CDKL;5*	NM 186098	NP 910987
	Oryza sativa	*Orysa;CDKL;6*	XM 479002	XP 479002
	Oryza sativa	*Orysa;CDKL;7*	AK122094	-NA-
	Oryza sativa	*Orysa;CDKL;8*	AK105621	-NA-
	Oryza sativa	*Orysa;CDKL;9*	AK067238	-NA-
	Oryza sativa	*Orysa;CDKL;10*	XM 479750	XP 479750
	Oryza sativa	*Orysa;CDKL;11*	AK072696	-NA-
	Oryza sativa	*Orysa;CDKL;12*	AK068916	-NA-
	Oryza sativa	*Orysa;CDKL;13*	XM 466234	XP 466234
	Oryza sativa	*Orysa;CDKL;14*	XM 466235	XP 466235
	Oryza sativa	*Orysa;CDKL;15*	AK121206	-NA-
	Oryza sativa	*Orysa;CDKL;16*	AK100360	-NA-
	Oryza sativa	*Orysa;CDKL;17*	AK100088	-NA-
	Oryza sativa	*Orysa;CDKL;18*	XM 463674	XP 463674

Formation of CDK-cyclin complex

Cyclin is a protein that contains a conserved region of 250 amino acids called the cyclin core (Pines 1995). It consists of two domains: cyclin N and cyclin C (Nugent *et al.* 1991). The cyclin C domain is less conserved and is present in most but not all cyclins, signifying a specific but not a critical function of this domain. The cyclin N domain has the CDK-binding region, which is 100 amino acids long called cyclin box and is the defining domain for cyclins. Evans *et al*. 1983 discovered cyclins in sea urchin eggs and suggested the biochemical mechanism that drives the cell cycle. Earlier only one pair of cyclins, which coordinated S phase (A-type) and M phase (B-type), was known. But later it was shown that it is only one kinase subunit (Lohka *et al.* 1988). Large group of cyclins, which are specific for plants and animals, are known. In plants more than 100 cyclins have been isolated from various species. There are three major classes of plant cyclins involved in cell cycle regulation, A-type cyclins (CYCA), B-type cyclins (CYCB), and D-type cyclins (CYCD). CYCD/CDK complexes control the G1/S transition by inhibiting the retinoblastoma-related protein (RBR), resulting in the transcription of genes involved in DNA replication. CYCB/CDK complexes control the G2/M transition. The role of CYCA/CDK complexes is less clear; they appear to play various roles in maintaining S phase and in the G2/M transition (Churchman *et al.* 2006).

D-type cyclins are the primary sensors of external conditions that control the first checkpoint in plants and direct phosphorylation of the plant homologue to the

retinoblastoma protein (RBR; De Jager *et al.* 2005, Uemukai *et al.* 2005). RBR is rate limiting for S-phase entry (Menges *et al.* 2006). RBR also participates in controlling cell differentiation as its deficiency delays cell differentiation (Desvoyes *et al.* 2006). On the other hand, its induced expression leads meristematic cells into the differentiation pathway (Wyrzykowska *et al.* 2006). Cell cycle entry is characterized by the activation of A-type cyclin dependent kinase (CDKA) that forms complex with D-type cyclin (CYCD) and phosphorylates the RBR protein. Thus RBR and histone deacetylases (HDAC) proteins are released from the E2F-DP transcription factors (De Veylder *et al.* 2007). Thus, G1-S cyclins respond to external signals and control growth rate or cell size. In short we can say that D-type cyclins regulate G1-to-S transition, A-type cyclins regulate S-to-M phase control, and B-type cyclins regulates both the G2-to-M transition and intra-M-phase control (Breyne and Zabeau 2001).

CDK is inactive without cyclin association, so that regulated synthesis, destruction and localization of the cyclin is required for multiple level of control over activation of the CDK enzymes. They form complex with a number of different CDKs. This association of CDK and cyclin is a key for regulation of serine-threonine protein kinases. The basic principle of the cell cycle oscillator is the regulated synthesis and destruction of cyclins that provides waves to CDK enzymes for activity, which in turn drive the two crucial transitions of the cell cycle from G1 into S phase and from G2 into M phase.

Inhibition of CDK activity

During cell division certain specific protein are synthesized, which interact with CDKs to terminate their function. CDK activity can be terminated by binding, phosphorylation or dephosphorylation of these proteins (Morgan 1997). The first plant CDK inhibitor gene was identified from *Arabidopsis* (Wang *et al.* 1997). CDK inhibitors isolated so far from plants contain a C-terminal domain that shares similarity with the mammalian Cip/Kip CDK inhibitors in a region of about 30 residues important for CDK inhibition (Wang *et al.* 1997). Seven CDK inhibitor genes are identified from *Arabidopsis* (Wang *et al.* 1997, Lui *et al.* 2000, De Veylder *et al.* 2001, Zhou *et al.* 2002). They were named as *ICK1* (interactor/inhibitor of CDK1; Wang *et al.* 1997), *ICK2* (interactor/inhibitor of CDK2; Lui *et al.* 2000), and *KRP3* to *KRP7* (Kip-related protein 1 to 7; De Veylder *et al.* 2001). ICK/KRP proteins consist of conserved C-terminal domain, which is similar to a domain in the N-terminal region of the mammalian Cip/Kip CDK inhibitors (Zhou *et al.* 2002). Genes similar to *ICK*/*KRP* genes have been identified from *Chenopodium rubrum* (Fountain *et al.* 1999), tobacco (Jasinski *et al.* 2002, 2003), maize (Coelho *et al.* 2005), alfalfa (Pettko-Szandtner *et al.* 2006) and tomato (Bisbis *et al.* 2006). *ICK/KRP*s genes showed different patterns of expression in leaf, root, and inflorescence and flower tissues of *Arabidopsis* (Wang *et al.* 1998, Lui *et al.* 2000, De Veylder *et al.* 2001). The expression of *ICK1* and *ICK2* is absent in shoot apical meristems and vascular cells while *KRP4* and *KRP5* are expressed mostly in proliferating cells (Ormenese *et al.* 2004). The different pattern of expression of *ICK*/*KRP* genes indicates functional differences among them during plant development. The plant ICK/KRP proteins interact with both D-type cyclins and A-type CDK or with D-type cyclins alone. *Arabidopsis ICK1*, *ICK2*, *KRP3* and *KRP4* interacts with *CDKA;1* while *KRP6* (*ICK4*) and *KRP7* (*ICK5*) shows no interaction (Zhou *et al.* 2002). *KRP6* and *KRP7* are more closely related to each other, based on conserved motifs and exon-intron organization. ICK/KRP proteins shows difference based on their ability to interact with the A-type CDK. However, plant CDK inhibitors do not interact with B-type as well as C-type CDKs (Bisbis *et al.* 2006, Pettko-Szandtner *et al.* 2006).

ICK/KRP proteins can inhibit CDK activity *in vitro* and *in vivo*. The ability to inhibit CDK activity has been shown for recombinant proteins of several ICK/KRPs from *Arabidopsis* (Wang *et al.* 1997, 1998, Lui *et al.* 2000), tobacco (Jasinski *et al.* 2002), maize (Coelho *et al.* 2005) and tomato (Bisbis *et al.* 2006). The level of inhibition is concentration dependent (Coelho *et al.* 2005, Bisbis *et al.* 2006). The C-terminal conserved domain is required for the interaction of ICK1 with the CDK complex and for inhibition of its activity in plants (Zhou *et al.* 2003). On the other hand, removal of the N-terminal region had no effect on the ability of the mutant ICK1109-191 protein to interact with and inhibit the CDK complex (Zhou *et al.* 2003). Thus, the C-terminal domain confers CDK inhibitory function to plant CDK inhibitors as in the animal Cip/Kip inhibitors.

Degradation of cyclin by ubiquitin-dependent proteolysis

Successful progression through cell cycle requires coordinated destruction of essential regulatory proteins by the UPS (Vodermaier 2004). Cyclins are typically unstable proteins so they rapidly get turnover by the ubiquitination-proteasome pathway (UPS). Degradation of cyclins *via* the UPS is a two-step process, in which the cyclin is firstly tagged by covalent attachment of ubiquitin and then degraded by a multicatalytic protease complex called the 26S proteasome. Conjugation of ubiquitin to the cyclin involves a cascade of three enzymes: E1, E2 and E3. Ubiquitin-activating enzyme (E1) forms a high-energy thioester intermediate,

E1-S~Ubi, which is then transesterified to one of the several ubiquitin-conjugating enzyme (E2). The transfer of ubiquitin from the E2-S~Ubi to an ε-NH2 group of an internal lysine residue in the target protein substrate is done by an ubiquitin protein ligase enzyme (E3). By adding activated ubiquitin moieties to internal lysine residues on the conjugated ubiquitin molecule, a polyubiquitin chain is synthesized. Polyubiquitin chains consist of at least four ubiquitin monomers, in which the C-terminus of one ubiquitin molecule is attached to the lysine 48 of the next ubiquitin molecule. This is recognized by the 26S proteasome. However, conjugated cyclins can still be deubiquitinated by deubiquitylating enzymes (DUBs) prior to proteolysis. This is an important step in regulation process, as it is the last step to reverse cyclin degradation (Amerik and Hochstrasser 2004). At last, when ubiquitin-conjugated cyclins finally reach their destination, *i.e.*, 26S proteasome, they will be unfolded and get threaded into the cylindrical central part of 26S proteasome, where they are cleaved to peptides and the ubiquitin monomers are recycled (Hendil and Hartmann-Petersen 2004).

Recognization of specific cyclin by E3 ligase enzyme

The E1 enzyme activates ubiquitin in a thioester linkage, which is trans-esteried to a ubiquitin-conjugating E2 enzyme. Transfer of activated ubiquitin to a substrate lysine requires its recognition by an E3 ligase. The E3 enzymes specify the substrates and play the most important role in the ubiquitylation reaction. Two families E3 ligases dominate DNA duplication and cell division: the Skp1/cullin/F-box (SCF)-related complex and the APC/C (anaphase-promoting complex/ cyclosome). These E3 enzymes have been subdivided into two major classes: those that contain a HECT (homologous to the E6-AP C-terminus) domain and those that contain a RING domain (Pickart 2001). E3 enzymes request for a RING-H2 finger subunit that interacts with the E2 enzyme to stimulate the ubiquitylation reaction. In addition, these E3 enzymes bear substrate adaptor proteins that recruit specifically the substrates to the core complex. Substrate recognized by the SCF requests for phosphorylation of the target proteins. Finally, multi-ubiquitylated proteins are recognized by the 26S proteasome and proteolyzed into peptides, and ubiquitin is recycled through the action of deubiquitylating enzymes.

SCF regulating the G1/S transition

Cullins (CULs) are a family of hydrophobic proteins that act as scaffolds for ubiquitin ligases (E3). Each CUL protein forms a different class of E3 enzymes; among them, the best characterized complexes are the SCF (Skp1/CUL1/F-box; Cardozo and Pagano 2004), the ECS (ElonginC-CUL2-SOCS-box; Ivan and Kaelin 2001), the CUL3-BTB (Pintard *et al.* 2004) complexes and the APC/C (Peters 2002), which contains a more distant CUL member, called APC2. CUL1 forms are presently the best characterized cell cycle regulating enzyme (Cardozo and Pagano 2004). CUL1 interacts at C-terminus with the RING domain protein RBX1 (also called ROC1 or HRT1) and E2 enzyme and at N-terminus with the adaptor protein SKP1. F-box proteins contain a rather variable interaction domain known as the F-box that binds to SKP1. Additionally, F-box proteins carry a great variety of typical protein-protein interaction domains that confer substrate specificity for ubiquitylation.CUL1 is also regulated by the covalent linkage of an ubiquitin-like protein, RUB1/NEDD8, through the neddylation activating and conjugating enzymes (Hochstrasser 1998). NEDD8- modification of CUL1 dissociates CAND1, an inhibitor of the SCF, and consequently promotes the binding of SKP1 and the F-box protein to CUL1 and thus allows the formation of SCF E3 ligase complex (Liu *et al.* 2002). The neddylation of CUL1 is removed by the peptidase activity of the COP9-signalosome (CSN) in order to allow cell cycle to enter in S phase (Cope and Deshaies 2003).

APC/C regulating G2/M transition

APC/C is a major player in the metaphase to anaphase transition. The proteolytic events triggered by APC/C are required to release sister chromatid cohesion during anaphase, specify the exit from mitosis and prevent premature entry into S phase. The APC/C complex comprises: APC2, the distant member of the cullin family, and the RING-finger protein APC11. These two subunits interact with each other and with E2 ubiquitin-conjugating enzymes and the complex have been shown to be sufficient for catalytic activity *in vitro* but without substrate specificity (Tang *et al.* 2001). APC/C contains special subunits which should be phosphorylated during mitosis to activate the APC/C. The APC/C is activated in early mitosis through cyclin B/CDK1-dependent phosphorylation and the binding of its activator CDC20. APC10/DOC1 is characterized by the presence of a DOC domain, which is important for substrate recognition and for extending polyubiquitin chain on a substrate (Carroll

et al. 2005). In addition to its core components, the APC/C requires a member of the WD40 family for activity. The coactivator CDC20/FIZZY or CDH1/FIZZY-RELATED activates APC/C to ubiquitylated substrates. They contain characteristic destruction motifs: the D-box, the KEN-box, the A-box and the GxEN-box that help to recognize specific substrate to be ubiquitylated (Kramer *et al.* 2000). Two domains are thought to play roles in binding of these activating subunits to the APC/C: a short internal motif called the C-box (Schwab *et al.* 2001) and a C-terminal IR dipeptide (Vodermaier *et al.* 2003). CDC20/FZ and CDH1/FZR also contain WD40 repeats that bind directly to APC/C targets, and thus may serve as a bridge between enzyme and substrate (Kraft *et al.* 2005). CDC20/FZ activates APC/C during metaphase, whereas CDH1/FZR promotes APC/C activity for the exit of mitosis and during G1. APC/C is mainly required to induce progression and exit from mitosis by inducing proteolysis of different cell cycle regulators including PDS1/securin and cyclin B. The proteolytic events triggered by APC/C are required to release sister chromatid cohesion during anaphase, specify the exit from mitosis and prevent premature entry into S phase. During the entry into anaphase, the APC/C-dependent degradation of securin causes activation of separase and as a consequence cleavage of the cohesion complex, which allows sister chromatids to separate from each other. Proteolysis of securin is ensured by APC/CCDC20 before anaphase onset, but degradation is maintained until the end of G1 by APC/CCDH1 (Nasmyth 2001). Although plant orthologues of securin proteins have not yet been reported (these proteins are poorly conserved) inhibition of the proteasome during prophase blocks the cells in metaphase, indicating that securing orthologues do exist in plants (Genschik *et al.* 1998). The degradation of securins is especially significant in a mechanism called the spindle assembly checkpoint (Hoyt *et al.* 1991). To ensure balanced chromosome segregation and to avoid aneuploidy, this mechanism delays anaphase and causes exit from mitosis only when all the chromosomes are properly attached to the spindle by means of a specialized complex called the kinetochore (Gorbsky 2001). Unattached kinetochores serve as catalytic sites for the formation of the checkpoint complexes containing MAD2 and CDC20 (Yu 2002). An unattached kinetochore becomes a source of a diffusible inhibitor of the APC/C. Two checkpoint proteins with key functions are MAD2 (mitotic arrest deficient 2) and BUBR1 (budding uninhibited by benzimidazole R1), both of which bind to the CDC20, resulting in inhibition of the activation of APC/C complex (Fang 2002) and, thus, inhibition of G2 to M phase progression.

From overall studies it is concluded that CDKs are enzymes which determine cell to initiate cell division. They give signal to cell depending on the surrounding nutritional and environmental conditions. If conditions are favorable, then genes of CDKD and CDKF get activated and start phosphorylation processes. Depending on the surrounding environment they utilize specific substrate and induce cell division process in cell. If specific substrate for induction of cell division is available than CDKF activates CDKD genes and starts phosporylation. The product formed at the end of phosphorylation acts as substrate for CDKA. Different *CDKA* genes are activated throughout cell cycle depending on the substrate synthesized by CDKD and CDKF. These CDKA controls G1/S and G2/M transition. CDKA1 induce DNA replication by synthesizing substrate required for activation of CDKB1. CDKB1 is involved in DNA replication. At the end of DNA replication, CDKB1 synthesizes specific substrate which activates CDKA2 and CDKA2 makes the cell to enter into mitosis. During mitosis, CDKB2 is activated and maintains 2C ratio within the daughter cells. After mitosis, CDKC is activated and induces daughter cell elongation. At last CDKD activates CDKE and CDKG synergistically and induces cell differentiation. Hence, CDKD and CDKF play an important role in growth and differentiation by controlling the overall level of CDK activity.

References

Amerik, A., Hochstrasser, M.: Mechanism and function of deubiquitinating enzymes. - Biochim. biophys. Acta **1695**: 189-207, 2004.

Bisbis, B., Delmas, F., Joube`s, J., Sicard, A., Hernould, M., Inze, D., Mouras, A., Chevalier, C.: Cyclin-dependent kinase (CDK) inhibitors regulate the CDK-cyclin complex activities in endoreduplicating cells of developing tomato fruit. - J. biol. Chem. **281**: 7374-7383, 2006.

Bögre, L., Zwerger, K., Meskiene, I., Binarova, P., Csizmadia, V., Planck, C., Wagner, E., Hirt, H., Heberle-Bors, E.: The cdc2Ms kinase is differentially regulated in the cytoplasm and in the nucleus. - Plant. Physiol. **113**: 841-852, 1997.

Boruc, J., Mylle, E., Duda, M., De Clercq, R., Rombauts, S., Geelen, D., Hilson, P., Inze, D., Van Damme, D., Russinova, E.: Systematic localization of the *Arabidopsis* core cell cycle proteins reveals novel cell division complexes. - Plant Physiol. **152**: 553-565, 2010.

Boudolf, V., Barrôco, R., De Almeida Engler, J., Verkest, A., Beeckman, T., Naudt, M., Inzé, D., De Veylder, L.: B1 type cyclin-dependent kinases are essential for the formation of stomatal complexes in *Arabidopsis thaliana*. - Plant Cell **16**: 945-955, 2004a.

Boudolf, V., Vlieghe, K., Beemster, G.T.S., Magyar, Z., Torres Acosta, J.A., Maes, S., Van Der Schueren, E., Inzé, D., De Veylder, L.: The plant-specific cyclin-dependent kinase CDKB1;1 and transition factor E2Fa-DPa control the balance of mitotically dividing and endoreduplicating cells in *Arabidopsis*. - Plant Cell **16**: 2683-2692, 2004b.

Breyne, P., Zabeau M.: Genome-wide expression analysis of plant cell cycle modulated genes. - Curr. Opin. Plant Biol.

4:136-42, 2001.
Cardozo, T., Pagano, M.: The SCF ubiquitin ligase: insights into a molecular machine. - Nat. Rev. mol. cell. Biol. **5**: 739-751, 2004.
Carroll, C.W., Enquist-Newman, M., Morgan, D.O.: The APC subunit Doc1 promotes recognition of the substrate destruction box. - Curr. Biol. **15**:11-18, 2005.
Churchman, M.L., Brown, M.L., Kato, N., Kirik, V., Hülskamp, M., Inzé, D., De Veylder, L., Walker, J.D., Zheng, Z., Oppenheimer, D.G., Gwin, T., Churchman, J., Larkin, J.C.: SIAMESE: a plant-specific cell cycle regulator, controls endoreduplication onset in *Arabidopsis thaliana*. - Plant Cell **18**: 3145-3157, 2006.
Coelho, C.M., Dante, R.A., Sabelli, P.A., Sun, Y., Dilkes, B.P., Gordon-Kamm, W.J., Larkins B.A.: Cyclin-dependent kinase inhibitors in maize endosperm and their potential role in endoreduplication. - Plant Physiol. **138**: 2323-2336, 2005.
Cope, G.A., Deshaies, R.J.: COP9 signalosome: a multifunctional regulator of SCF and other cullin-based ubiquitin ligases. - Cell **114**: 663-671, 2003.
Criqui, M.C., Weingartner, M., Capron, A., Parmentier, Y., Shen, W.H., Heberle- Bors, E., Bögre, L., Genschik, P.: Sub-cellular localisation of GFP tagged tobacco mitotic cyclins during the cell cycle and after spindle checkpoint activation - Plant. J. **28**: 569-581, 2001.
De Jager, S.M., Maughan, S., Dewitte, W., Scofield, S., Murray, J.A.: The developmental context of cell-cycle control in plants. - Semin. Cell. Dev. Biol. **16**: 385-396, 2005.
De Veylder, L., Beeckman, T., Beemster, G.T.S., Krols, L., Terras, F., Landrieu, I., Van der Schueren, E., Maes, S., Naudts, M., Inze, D.: Functional analysis of cyclin-dependent kinase inhibitors of *Arabidopsis*. - Plant Cell. **13**: 1653-1668, 2001.
De Veylder, L., Beeckman, T., Inze, D.: The ins and outs of the plant cell cycle. - Natur. Rev. mol. cell. Biol. **8**: 655-665, 2007.
Desvoyes, B., Ramirez-Parra, E., Xie, Q., Chua, N.H., Gutierrez, C.: Cell type-specific role of the retinoblastoma/E2F pathway during Arabidopsis leaf development. - Plant Physiol. **140**: 67-80, 2006.
Dissmeyer, N., Nowack, M.K., Pusch, S., Stals, H., Inzé, D., Grini, P.E., Schnittger, A.: T-loop phosphorylation of *Arabidopsis* CDKA;1 is required for its function and can be partially substituted by an aspartate residue. - Plant Cell **19**: 972-985, 2007.
Evans, T., Rosenthal, E.T., Youngblom, J., Distel, D., Hunt, T.: Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. - Cell **33**: 389-396, 1983.
Fang, G.: Checkpoint protein BubR1 acts synergistically with Mad2 to inhibit anaphase promoting complex. - Mol. Biol. Cell **13**: 755-766, 2002.
Fountain, M.D., Renz, A., Beck, E.: Isolation of a cDNA encoding a G1-cyclin-dependent kinase inhibitor (ICDK) from suspension cultured photoautotrophic *Chenopodium rubrum* L. cells. (accession No. AJ002173) (PGR 99-057). - Plant Physiol. **120**: 339-340, 1999.
Fulop, K., Pettko-Szandtner, A., Magyar, Z., Miskolczi, P., Kondorosi, E., Dudits, D., Bako, L.: The *Medicago* CDKC;1-CYCLINT;1 kinase complex phosphorylates the carboxy-terminal domain of RNA polymerase II and promotes transcription. - Plant J. **42**: 810-20, 2005.
Genschik, P., Criqui, M.C., Parmentier, Y., Derevier, A., Fleck, J.: Cell cycle-dependent proteolysis in plants: Identification of the destruction box pathway and metaphase arrest produced by the proteasome inhibitor MG132. - Plant Cell. **10**: 2063-2076, 1998.
Gorbsky, G.J.: The mitotic spindle checkpoint. - Curr. Biol. **11**: R1001-R1004, 2001.
Hemerly, A., De Almeida Engler, J., Bergounioux, C., Van Montagu, M., Engler, G., Inzé, D., Ferreira, P.: Dominant negative mutants of the CDC2 kinase uncouple cell division from iterative plant development. - EMBO J. **14**: 3925-3936, 1995.
Hendil, K.B., Hartmann-Petersen, R.: Proteasomes: a complex story. - Curr. Protein Peptide Sci. **5**: 135-151, 2004.
Hirayama, T., Imajuku, Y., Anai, T., Matsui, M., Oka, A.: Identification of two cell-cycle controlling cdc2 gene homologs in *Arabidopsis thaliana*. - Gene **105**:159-65, 1991.
Hirt, H., Páy, A., Bögre, L., Meskiene, I., Heberle-Bors, E.: cdc2MsB a cognate cdc2 gene from alfalfa, complement the G1/S but not the G2/M transition of budding yeast cdc28 mutants. - Plant J. **4**: 61-69, 1993.
Hochstrasser, M.: There's the rub: a novel ubiquitin-like modification linked to cell cycle regulation. - Genes Dev. **12**: 901-907, 1998.
Hoyt, M.A., Totis, L., Roberts, B.T.: *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. - Cell **66**: 507-517, 1991.
Inzé, D., De Veylder, L.: Cell cycle regulation in plant development. - Annu. Rev. Genet. **40**: 77-105, 2006.
Ivan, M., Kaelin, W.G, Jr.: The von Hippel-Lindau tumor suppressor protein. - Curr. Opin. Genet. **11**: 27-34, 2001.
Jasinski, S., Leite, C.S., Domenichini, S., Stevens, R., Raynaud, C., Perennes, C., Bergounioux, C., Glab, N.: NtKIS2, a novel tobacco cyclin-dependent kinase inhibitor is differentially expressed during the cell cycle and plant development. - Plant Physiol. Biochem. **41**: 667-676, 2003.
Jasinski, S., Perennes, C., Bergounioux, C., Glab, N.: Comparative molecular and functional analyses of the tobacco cyclin-dependent kinase inhibitor NtKIS1a and its spliced variant NtKIS1b1. - Plant Physiol. **130**: 1871-1882, 2002.
Joubes, J., Chevalier, C., Dudits, D., Heberle-Bors, E., Inze, D., Umeda, M., Renaudin, J.: CDK-related protein kinases in plants. - Plant mol. Biol. **43**: 607-620, 2000.
Kraft, C., Vodermaier, H.C., Maurer-Stroh, S., Eisenhaber, F., Peters, J.M.: The WD40 propeller domain of Cdh1 functions as a destruction box receptor for APC/C substrates. - Mol. Cell. **18**: 543-553, 2005.
Kramer, E.R., Scheuringer, N., Podtelejnikov, A.V., Mann, M., Peters, J.M.: Mitotic regulation of the APC activator proteins CDC20 and CDH1. - Mol. Biol. Cell **11**:1555-1569, 2000.
Lee, J., Das, A., Yamaguchi, M., Hashimoto, J., Tsutsumi, N., Uchimiya, H., Umeda, M.: Cell cycle function of a rice B2-type cyclin interacting with a B-type cyclin-dependent kinase. - Plant J. **34**: 417-425, 2003.
Liu, J., Furukawa, M., Matsumoto, T., Xiong, Y.: NEDD8 modification of CUL1 dissociates p120CAND1, an inhibitor of CUL1-SKP1 binding and SCF ligases. - Mol. Cell **10**: 1511-1518, 2002.
Lohka, M.J., Hayes, M.K., Maller, J.L.: Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. - Proc. nat. Acad. Sci. USA **85**: 3009-3013, 1988.
Lui, H., Wang, H., DeLong, C., Fowke, L.C., Crosby, W.L., Fobert, P.R.: The *Arabidopsis* Cdc2a-interacting protein

ICK2 is structurally related to ICK1 and is a potent inhibitor of cyclin-dependent kinase activity *in vitro*. - Plant J. **21**: 379-385, 2000.

Magyar, Z., Mészáros, T., Miskolczi, P., Deák, M., Fehér, A., Brown, S., Kondorosi, E., Athanasiadis, A., Pongor, S., Bilgin, M., Bakó, L., Koncz, C., Dudits, D.: Cell cycle phase specificity of putative cyclin dependent kinase variants in synchronized alfalfa cells. - Plant Cell **9**: 223-235, 1997.

Maldonado, E., Shiekhattar, R., Sheldon, M., Cho, H., Drapkin, R., Rickert, P., Lees, E., Anderson, C.W., Linn, S., Reinberg, D.: A human RNA polymerase II complex associated with SRB and DNA-repair proteins. - Nature **381**: 86-89, 1996.

Menges, M., De Jager, S.M., Gruissem, W., Murray, J.A.H.: Global analysis of the core cell cycle regulators of *Arabidopsis* identities novel genes, reveals multiple and highly specific profiles of expression and provides a coherent model for plant cycle control. - Plant J. **41**: 546-566, 2005.

Menges, M., Samland, A.K., Planchais, S., Murray, J.A.: The D-type cyclin CYCD3;1 is limiting for the G1-to-S-phase transition in *Arabidopsis*. - Plant Cell **18**: 893-906, 2006.

Meszaros, T., Miskolczi, P., Ayaydin, F., Pettkó-Szandtner, A., Peres, A., Magyar, Z., Horváth, G.V., Bakó, L., Fehér, A., Dudits, D.: Multiple cyclin-dependent kinase complexes and phosphatases control G2/M progression in alfalfa cells. - Plant mol. Biol. **43**: 595-605, 2000.

Mews, M., Sek, F.J., Volkmann, D., John, P.C.L.: Immunodetection of four mitotic cyclins and the Cdc2a protein kinase in the maize root: their distribution in cell development and dedifferentiation. - Protoplasma **212**: 236-249, 2000.

Morgan, D.O.: Cyclin-dependent kinases: engines, clocks, and microprocessors. - Annu. Rev. cell. dev. Biol. **13**: 261-291, 1997.

Nasmyth, K.: Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. - Annu. Rev. Genet. **35**: 673-745, 2001.

Novak, B., Csikasz-Nagy, A., Gyorffy, B., Chen, K., Tyson, J.J.: Mathematical model of the fission yeast cell cycle with checkpoint controls at the G1/S, G2/M and metaphase/anaphase transitions. - Biophys. Chem. **72**: 185-200, 1998.

Nugent, J.H., Alfa, C.E., Young, T., Hyams, J.S.: Conserved structural motifs in cyclins identified by sequence analysis. - J. cell. Sci. **99**: 669-674, 1991.

Ormenese, S., Engler, J.D., De Groodt, R., De Veylder, L., Inzé, D., Jacqmard, A.: Analysis of the spatial expression pattern of seven Kip related proteins (KRPs) in the shoot apex of *Arabidopsis thaliana*. - Ann. Bot. **93**: 575-580, 2004.

Peters, J.M.: The anaphase-promoting complex: proteolysis in mitosis and beyond. - Mol. Cell **9**: 931-943, 2002.

Pettko-Szandtner, A., Mészaros, T., Horvath, G.V., Bakó, L., Csordás-Tóth, E., Blastyák, A., Zhiponova, M., Miskolczi, P., Dudits, D.: Activation of an alfalfa cyclin-dependent kinase inhibitor by calmodulin-like domain protein kinase. - Plant J. **46**: 111-123, 2006.

Pickart, C.M.: Mechanism underlying ubiqutination. - Annu. Rev. Biochem. **70**: 503-533, 2001.

Pines, J.: Cyclins and cyclin-dependent kinases: a biochemical view. - Biochem. J. **308**: 697-711, 1995.

Pintard, L., Willems, A., Peter, M.: Cullin-based ubiquitin ligases: Cul3-BTB complexes join the family. - EMBO. J. **23**: 1681-1687, 2004.

Porceddu, A., Stals, H., Reichheld, J., Segers, G., De Veylder, L., Barrôco, R.D., Casteels, P., Van Montagu, M., Inzé, D., Mironov, V.: A plant-specific cyclin-dependent kinase is involved in the control of G2/M progression in plants. - J. biol. Chem. **276**: 36354-36360, 2001.

Rickert, P., Seghezzi, W., Shanahan, F., Cho, H., Lees, E.: Cyclin C/CDK8 is a novel CTD kinase associated with RNA polymerase II. - Oncogene **12**: 2631-2640, 1996.

Schwab, M., Neutzner, M., Mocker, D., Seufert, W.: Yeast Hct1 recognizes the mitotic cyclin Clb2 and other substrates of the ubiquitin ligase APC. - EMBO J. **20**: 5165-5175, 2001.

Segers, G., Gadisseur, I., Bergounioux, C., De Almeida, E.J., Jacqmard, A., Van Montagu, M., Inze, D.: The *Arabidopsis* cyclin-dependent kinase gene *cdc2bAt* is preferentially expressed during S and G2 phases of the cell cycle. - Plant J. **10**: 601-612, 1996.

Stals, H., Bauwens, S., Traas, J., Van Montagu, M., Engler, G., Inzé, D.: Plant CDC2 is not only targeted to the pre-prophase band, but is also co-located with the spindle, phragmoplast, and chromosomes. - FEBS Lett. **418**: 229-234, 1997.

Tang, Z., Li, B., Bharadwaj, R., Zhu, H., Özkan, E., Hakala, K., Deisenhofer, J., Yu, H.: APC2 cullin protein and APC11 ring protein comprise the minimal ubiquitin ligase module of the anaphase-promoting complex. - Mol. Biol. Cell **12**: 3839-3851, 2001.

Uemukai, K., Iwakawa, H., Kosugi, S., De Uemukai, S., Kato, K., Kondorosi, E., Murray, J.A., Ito, M., Shinmyo, A., Sekine, M.: Transcriptional activation of tobacco E2F is repressed by co-transfection with the retinoblastoma-related protein: cyclin D expression overcomes this repressor activity. - Plant mol. Biol. **57**: 83-100, 2005.

Umeda, M., Shimotohno, A., Yamaguchi, M.: Control of cell division and transcriptionby cyclin-dependent kinase-activating kinases in plants. - Plant Cell Physiol. **46**: 1437-1442, 2005.

Vodermaier, H.C.: APC/C and SCF: controlling each other and the cell cycle. - Curr. Biol. **14**: R787-R796, 2004.

Vodermaier, H.C., Gieffers, C., Maurer-Stroh, S., Eisenhaber, F., Peters, J.M.: TPR subunits of the anaphase-promoting complex mediate binding to the activator protein CDH1. - Curr. Biol. **13**: 1459-1468, 2003.

Wang, H., Fowke, L.C., Crosby, W.L.: A plant cyclin-dependent kinase inhibitor gene. - Nature **386**: 451-452, 1997.

Wang, H., Qi, Q., Schorr, P., Cutler, A.J., Crosby, W.L., Fowke, L.C.: ICK1, a cyclin dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and CYCD3, and its expression is induced by abscisic acid. - Plant J. **15**: 501-510, 1998.

Wang, W., Chen, X.: Hua Enhancer3 reveals a role for a cyclin-dependent protein kinase in the specification of floral organ identity in *Arabidopsis*. - Development **131**: 3147-3156, 2004.

Wyrzykowska, J., Schorderet, M., Pien, S., Gruissem, W., Fleming, A.J.: Induction of differentiation in the shoot apical meristem by transient overexpression of a retinoblastoma-related protein. - Plant Physiol. **141**: 1338-1348, 2006.

Yu, H.: Regulation of APC-Cdc20 by the spindle checkpoint. - Curr. Opin. cell. Biol. **14**: 706-714, 2002.

Zhiponova, M.K., Pettko-Szandtner, A., Stelkovics, É., Neer, Z., Bottka, S., Krenacs, T., Dudits, D., Feher, A., Szilak L.: Mitosis-specific promoter of the alfalfa cyclin-dependent kinase gene (Medsa;CDKB2;1) is activated by wounding

and ethylene in a non-cell division-dependent manner. - Plant physiol. **140**: 693-703, 2006.

Zhou, Y., Li, G., Brandizzi, F., Fowke, L.C., Wang, H.: The plant cyclin-dependent kinase inhibitor ICK1 has distinct functional domains for *in vivo* kinase inhibition, protein instability and nuclear localization. - Plant J. **35**: 476-489, 2003.

Zhou, Y., Fowke, L.C., Wang, H.: Plant CDK inhibitors: studies of interactions with cell cycle regulators in the yeast two-hybrid system and functional comparisons in transgenic *Arabidopsis* plants. - Plant Cell Rep. **20**: 967-975, 2002.