Plant Biotechnology 23, 211–214 (2006)

Gene Note

Isolation and characterization of a rice cDNA encoding B1-type cyclin-dependent kinase

Norihiro Sakaguchi[1,2], Tomoyuki Furukawa[3], Hiroaki Shimada[4], Junji Hashimoto[5], Kengo Sakaguchi[2], Masaaki Umeda[1]*

[1] Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan; [2] Department of Applied Biological Science, Faculty of Science and Technology, Tokyo University of Science, Noda-shi, Chiba 278-8510, Japan; [3] Tissue Engineering Research Center, Tokyo University of Science, Noda-shi, Chiba 278-8510, Japan; [4] Department of Biological Science and Technology, Tokyo University of Science, Noda-shi, Chiba 278-8510, Japan; [5] National Institute of Agrobiological Sciences, Tsukuba-shi, Ibaraki 305-8602, Japan
* E-mail: mumeda@iam.u-tokyo.ac.jp Tel: +81-3-5841-7845 Fax: +81-3-5841-8466

Received November 14, 2005; accepted November 30, 2005 (Edited by T. Kohchi)

Abstract Cyclin-dependent kinases (CDKs) are central players that control the cell cycle. In plants, A- and B-type CDKs are directly involved in cell cycle regulation. B-type CDK (CDKB) is unique to plants; however, only limited information on this kinase has been accumulated thus far. In the present study, we identified a rice cDNA encoding CDKB1;1 and studied its expression in suspension-cultured cells and plant tissues. We found that this enzyme was expressed in actively dividing cells in suspension cultures and was downregulated by the depletion of sucrose from the medium. In plants, the *CDKB1;1* transcripts were highly expressed in the shoot and root apical meristems, but not in mature plant organs. These results suggested that CDKB1 is mainly involved in mitotic cell division during plant development.

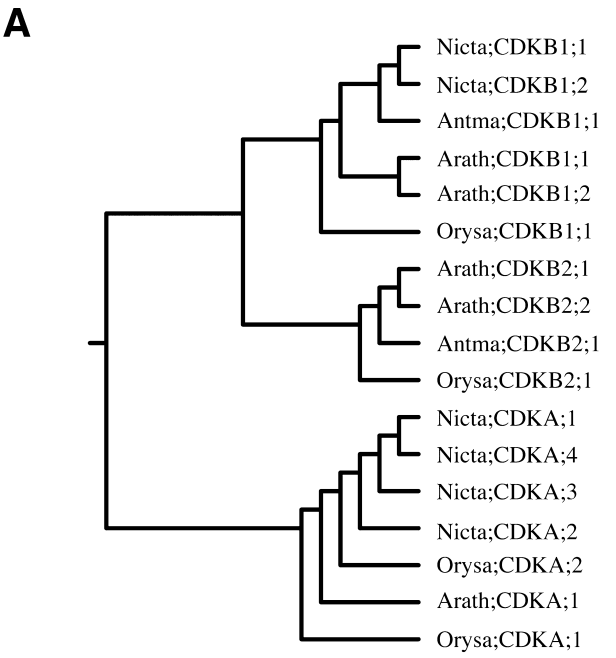
Key words: CDK, cell cycle, cyclin, *Oryza sativa*, rice.

The eukaryotic cell cycle is controlled by cyclin-dependent serine/threonine protein kinases (CDKs). In yeasts, a single CDK binds to cyclins and regulates the progression through each cell cycle phase. In contrast, animals have distinct types of CDKs that regulate specific cell cycle phases by forming complexes with specific cyclin partners (Morgan 1997). These CDKs are characterized by the PSTAIRE motif that is responsible for the binding of cyclins (Jeffrey et al. 1995; Morgan 1996). Plants also have multiple types of CDKs including CDKA containing the PSTAIRE motif and the plant-specific CDKB containing altered PSTAIRE sequences such as PPTTLRE or PPTALRE (Joubès et al. 2000). CDKBs are further divided into two subclasses, namely, CDKB1 and CDKB2, on the basis of sequence relationships. Although the transcript and protein levels of CDKA remain constant throughout the cell cycle, the expression of *CDKB* depends on the cell cycle phases. Recent reports showed that *CDKB1* is expressed from the late S phase to the early M phase, while *CDKB2* is expressed in a narrow window during progression from the G2 to the M phase (Umeda et al. 1999; Meszaros et al. 2000; Menges and Murray 2002; Oakenfull et al. 2002).

In rice plants, three cDNAs encoding CDKA;1, CDKA;2, and CDKB2;1, respectively, have been isolated. *In situ* hybridization analysis showed that the gene for any one of these enzymes was expressed in the dividing cells of the root meristem, while the transcripts of *CDKA;1* also accumulated in differentiated cells such as those of the sclerenchyma, pericycle, and parenchyma of the central cylinder (Umeda et al. 1999). Recently, it was reported that rice CDKB2;1 interacts with cyclin B2 (CycB2) to form an active kinase complex (Lee et al. 2003). In rice plants, upregulation of CDKB2;1 by overexpression of *CycB2;2* resulted in accelerated root growth. In contrast, no information regarding the rice B1-type CDKs has been reported thus far. In the present study, we identified a cDNA encoding rice CDKB1 and analyzed its expression pattern in suspension-cultured cells and plant tissues.

In order to identify rice cDNAs encoding CDK homologues, we searched the EST database of *Oryza sativa* L. var. Nipponbare. It was found that the EST clone C11876 contains an open reading frame that encodes a predicted product of 34.6 kDa that is significantly similar to plant CDKs. This clone was identified as a B1-type CDK by phylogenetic analysis and showed the closest similarity to *Arabidopsis* CDKB1;1, alfalfa CDKB1;1 and *Antirrhinum* CDKB1;1

Abbreviations: CDK, cyclin-dependent kinase; cDNA, complementary DNA; EST, expression sequence tag; PCNA, proliferative cell nuclear antigen.
This article can be found at http://www.jspcmb.jp/



B

Arath;CDKB1;1	38	EMDEEGI**PPTALRE**ISLLQMLSTS	61
Arath;CDKB1;2	38	EMDEEGI**PPTALRE**ISLLQMLSQS	61
Antma;CDKB1;1	38	EMDEEGV**PPTALRE**VSLLQMLSQS	61
Medsa;CDKB1;1	38	EMDEEGV**PPTALRE**VSLLQMLSQS	61
Orysa;CDKB1;1	38	EMDEEGI**PPTALRE**ISILRLLSQS	61
Arath;CDKB2;1	48	HEDEEGV**PSTTLRE**ISILRMLARD	71
Arath;CDKB2;2	49	HEDEEGV**PPTTLRE**ISILRMLARD	72
Antma;CDKB2;1	48	HEDEEGV**PPTTLRE**VSLLRMLSRD	71
Medsa;CDKB2;1	52	HEDDEGV**PPTTLRE**VSILRMLSRD	75
Orysa;CDKB2;1	38	PEDDEGV**PPTALRE**VSLLRMLSQD	61

Figure 1. Amino acid similarity of plant CDKs. (A) Phylogenetic tree obtained by amino acid sequence comparison of plant A- and B-type CDKs. The clustering was performed by using the program CLUSTAL W. (B) Alignment of amino acid sequences of plant CDKBs in the PSTAIRE region. The altered PSTAIRE sequences are indicated by bold letters. Numbers indicate amino acid positions. Antma, *Antirrhinum majus*; Arath, *Arabidopsis thaliana*; Medsa, *Medicago sativa*; Nicta, *Nicotiana tabacum*; Orysa, *Oryza sativa*.

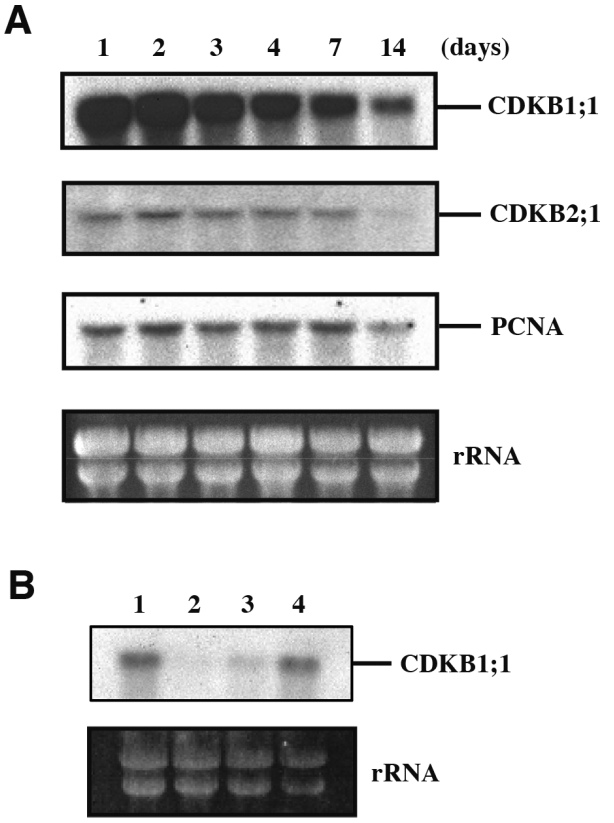


Figure 2. Expression of *CDKB1;1* in rice suspension-cultured cells. (A) Transcript levels at different time points after subculture. Total RNA samples (20 μg) were subjected to northern blotting. PCNA, which is expressed in actively dividing cells, was used as a control. (B) Transcript levels in sucrose-starved cells. Lane 1, 6-day-old cells cultured with sucrose; lane 2, 6-day-old cells cultured without sucrose; lane 3, 10-day-old cells cultured without sucrose; lane 4, cells cultured without sucrose for 6 days and then cultured with sucrose for 4 days. rRNA visualized with ethidium bromide is shown as a control.

(Figure 1A). The PPTALRE motif of the clone was present in the cyclin binding region (Figure 1B). Further, it is notable that rice CDKB2;1 has the PPTALRE sequence, while CDKB2 from other plant species contains the PPTTLRE motif (Figure 1B). This indicates that the PSTAIRE motif is not a characteristic of B1- and B2-type CDKs. We designated this clone *Orysa; CDKB1;1* according to the nomenclature proposed by Joubès et al. (2000). The nucleotide sequence data will be deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases under the Accession No. AB239918.

We analyzed the transcript level of *Orysa; CDKB1;1* (hereafter referred to as *CDKB1;1*) in rice suspension Oc cells (Baba et al. 1986). Cells (7-days-old) were diluted in a fresh medium and cultured for 1, 2, 3, 4, 7, and 14 days. Twenty micrograms of the total RNA was resolved on a 1.2% formaldehyde agarose gel and was subsequently transferred onto a nylon membrane (Hybond-N; Amersham Biosciences). After prehybridization, the membrane was probed with a ^{32}P-labeled cDNA of *CDKB1;1, CDKB2;1* (Umeda et al. 1999), or *PCNA* (Kimura et al. 2001) at 42°C for 16 h. Following this, it was washed twice with 2SSC and 0.1% SDS at room temperature for 15 min and three times with 0.1SSC and 0.1% SDS at 65°C for 20 min. Note that *CDKB1;1* is the sole gene for CDKB1 in rice plants, indicating the specificity of hybridization signals. *CDKB1;1* was highly expressed until day 2, and the transcript level gradually declined afterwards (Figure 2A). In contrast, *CDKB2;1* was expressed at almost the same level until day 7; however, the transcript was barely detected on day 14 (Figure 2A). This result suggests that *CDKB1;1* expression may be upregulated during the early phase of the cell culture.

We then analyzed the effect of sucrose starvation on *CDKB1;1* expression. Rice suspension cells were either cultured without sucrose for 6 or 10 days, or cultured for 6 days without sucrose followed by culture in a sucrose-

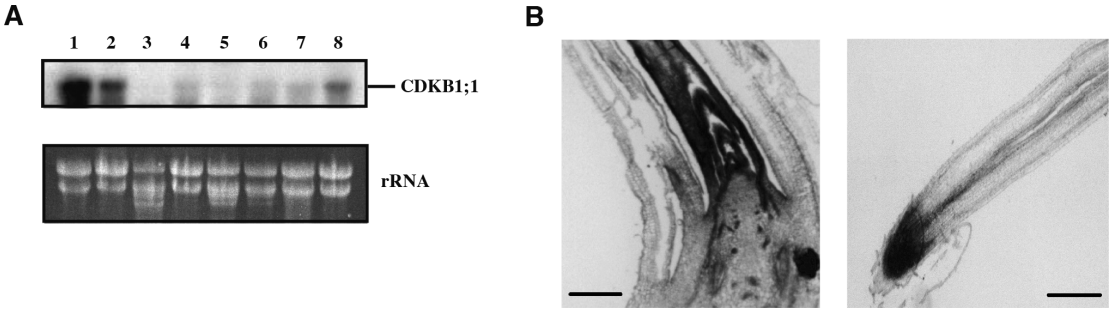


Figure 3. Expression of *CDKB1;1* in rice tissues. (A) Transcript levels in different rice tissues. Total RNA samples (20 μg) were subjected to northern blotting. Lane 1, suspension-cultured cells; lane 2, shoot apices including the shoot apical meristem; lane 3, mature leaves; lane 4, young leaves; lane 5, flag leaves; lane 6, panicles; lane 7, roots; lane 8, root tips including the root apical meristem. Each tissue was isolated from 50-day-old rice plants. rRNA visualized with ethidium bromide is shown as a control. (B) *In situ* hybridization of rice shoot (left) and root (right) apices with the probe specific for transcripts of *CDKB1;1*. Longitudinal sections were prepared from 10-day-old seedlings and allowed to hybridize with digoxigenin-labeled RNA probes. Hybridization signals are visible as purple staining. Bar = 100 μm.

containing medium for 4 days. When the rice cells were cultured without sucrose, the *CDKB1;1* transcripts almost disappeared after 6 or 10 days (Figure 2B). When the sucrose-starved cells were cultured in a sucrose-containing medium for 4 days, *CDKB1;1* expression was recovered, as shown in Figure 2B. These results indicate that *CDKB1;1* expression is tightly correlated with cell proliferation in suspension cultures.

In order to examine *CDKB1;1* expression in various organs, northern hybridization analysis was performed using the total RNA from 50-day-old rice plants. A high level of transcripts was detected in the shoot and root apices, whereas a lower expression was observed in young leaves, panicles, and roots (Figure 3A). No hybridization signal was detected in mature leaves (Figure 3A). This suggests that *CDKB1;1* is expressed in tissues containing proliferating cells. To further examine its expression in meristems, *in situ* hybridization was conducted with the shoot and root apices. Antisense RNA of *CDKB;1* was prepared from the cDNA and was labeled with digoxigenin by using a digoxigenin RNA labeling kit (Roche) according to the manufacturer's protocol. *In situ* hybridization was performed as described by Umeda et al. (1999). When digoxigenin-labeled sense RNA was used as a probe, no hybridization signal was detected (data not shown). The antisense probe showed strong signals in the shoot apical meristem and young leaves, but not in the mature leaves (Figure 3B). In the root apex, hybridization signals were localized in the root apical meristem and to a slight extent in the central cylinder (Figure 3B).

Together these data indicate that *CDKB1;1* expression is tightly linked to cell proliferation in meristems and other tissues that show cell division activity. Until now, only limited information has been obtained on B1-type CDKs in plants, particularly in the case of monocot plants. In *Arabidopsis*, *CDKB1;1* expression was observed in dividing cells associated with the formation of the stomatal complex (Boudolf et al. 2004). It was proposed that CDKB1;1 is involved in proper stomatal development. This suggests that CDKB1 may function in dividing cells wherein it controls mitotic cell division. This idea was supported by Porceddu et al. (2001) in which reported that the downregulation of CDKB1 activity caused G2/M delay; this indicates that CDKB1 functions in the control of the G2/M phase transition. Our results in monocotyledonous rice plants also support the idea that CDKB1 is mainly involved in mitosis, rather than in cell division competency as proposed for CDKA (Hemerly et al. 1993). It will be interesting to further unravel the interplay of cell division and differentiation in terms of A- and B-type CDK activities in plants.

Acknowledgments

We thank Mrs. Chikage Umeda-Hara for technical assistance in *in situ* hybridization experiments. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (grant no. 15031210) and a Grant-in-Aid for Scientific Research (B) (grant no. 16370019).

References

Baba A, Hasegawa S, Syono K (1986) Cultivation of rice protoplasts and their transformation mediated by *Agrobacterium* spheroplasts. *Plant Cell Physiol* 27: 463–471

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

Hemerly AS, Ferreira P, de Almeida Engler J, Van Montagu M, Engler G, Inzé D (1993) cdc2a expression in *Arabidopsis* is linked with competence for cell division. *Plant Cell* 5: 1711–1723

Jeffrey PD, Russo AA, Polyak K, Gibbs E, Hurwitz J, Massagué J, Pavletich NP (1995) Mechanism of CDK activation revealed by the structure of a cyclin A-CDK2 complex. *Nature* 376: 313–320

Joubès J, Chevalier C, Dudits D, Heberle-Bors E, Inzè D, Umeda

M, Renaudin JP (2000) CDK-related protein kinases in plants. *Plant Mol Biol* 43: 607–620

Kimura S, Suzuki T, Yanagawa Y, Yamamoto T, Nakagawa H, Tanaka I, Hashimoto J, Sakaguchi K (2001) Characterization of plant proliferating cell nuclear antigen (PCNA) and flap endonuclease-1 (FEN-1), and their distribution in mitotic and meiotic cell cycles. *Plant J* 28: 643–653

Lee J, Das A, Yamaguchi M, Hashimoto J, Tsutsumi N, Uchimiya H, Umeda M (2003) Cell cycle function of a rice B2-type cyclin interacting with a B-type cyclin-dependent kinase. *Plant J* 34: 417–425

Menges M, Murray JAH (2002) Synchronous *Arabidopsis* suspension cultures for analysis of cell-cycle gene activity. *Plant J* 30: 203–212

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

Morgan DO (1996) The dynamics of cyclin dependent kinase structure. *Curr Opin Cell Biol* 8: 767–772

Morgan DO (1997) Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu Rev Cell Dev Biol* 13: 261–291

Oakenfull EA, Riou-Khamlichi C, Murray JAH (2002) Plant D-type cyclins and the control of G1 progression. *Philos Trans R Soc Lond B Biol Sci* 357: 749–760

Porceddu A, Stals H, Reichheld J-P, Segers G, De Veylder L, de Pinho Barrôco R, Casteels P, Van Montagu M, Inzé D, Mironov V (2001) A plant-specific cyclin-dependent kinase is involved in the control of G_2/M progression in plants. *J Biol Chem* 276: 36354–36360

Umeda M, Umeda-Hara C, Yamaguchi M, Hashimoto J, Uchimiya H (1999) Differential expression of genes for cyclin-dependent protein kinases in rice plants. *Plant Physiol* 119: 31–40