Molecular cloning and mapping of casein kinase 2 alpha and beta subunit genes in barley

K. Kato, S. Kidou, and H. Miura

Abstract: Casein kinase 2 (CK2) is a ubiquitous, highly pleiotropic, constitutively active, and messenger-independent Ser/Thr protein kinase. It is found in two different forms: the heterotetrameric CK2, composed of two alpha catalytic subunits and two beta regulatory subunits, and the monomeric CK2 alpha, consisting of the alpha catalytic subunit. In the present study, we isolated barley cDNA clones of the CK2 alpha and beta subunit genes, designated *HvCK2A* and *HvCK2B*, respectively. Chromosome assignment, using a set of wheat–barley disomic chromosome addition lines, and RFLP mapping, using two doubled haploid populations, showed that *HvCK2A* was duplicated on the short arm of chromosome 2H and the long arm of chromosome 5H (designated *HvCK2a-2H* and *HvCK2a-5H*, respectively), and a single copy of *HvCK2B* was located on the long arm of chromosome 1H (designated *HvCK2b*). A PCR-Southern hybridization experiment demonstrated that the *HvCK2A* sequence originated from the *HvCK2a-5H* locus, showing that at least *HvCK2a-5H* was expressed. The present cDNA sequences and genomic organization of the two subunits will facilitate further functional analysis of CK2 in barley.

Key words: barley, casein kinase 2, cDNA cloning, RFLP mapping.

Résumé : La caséine kinase 2 (CK2) est une protéine kinase Ser/Thr ubiquiste, très pléiotropique, constitutive et qui ne dépend pas d'un messager. Elle est retrouvée sous deux formes différentes : (1) un hétérotétramère CK2 formé de deux sous-unités catalytiques alpha et de deux sous-unités régulatrices bêta, et (2) un monomère CK2 alpha formé uniquement de la sous-unité catalytique alpha. Dans le présent travail, les auteurs ont isolé des clones d'ADNc correspondant aux gènes codant pour les sous-unités CK2A et CK2B chez l'orge, lesquels gènes ont été nommés *HvCK2A* et *HvCK2B* respectivement. La cartographie chromosomique, à l'aide de lignées d'addition disomiques blé-orge, et la cartographie RFLP, chez deux populations de lignées haploïdes doublées, ont montré que *HvCK2A* est présent en deux exemplaires situés sur le bras court du chromosome 2H et sur le bras long du chromosome 5H. Ces deux gènes ont été désignés respectivement *HvCK2a-2H* et *HvCK2a-5H*. L'unique copie du gène *HvCK2B* a été assignée au chromosome 1H et a été désignée *HvCK2b*. Une hybridation Southern–PCR a révélé que la séquence *HvCK2A* provenait à l'origine du locus *HvCK2a-5H* et que ce locus est exprimé. Ces séquences d'ADNc et l'organisation génomique des deux sous-unités faciliteront une plus ample analyse fonctionnelle des CK2 chez l'orge.

Mots-clés : orge, caséine kinase 2, clonage d'ADNc, cartographie RFLP.

[Traduit par la Rédaction]

Introduction

Protein kinase CK2 (casein kinase 2; EC 2.7.11.1) is a serine/threonine phosphotransferase that occurs ubiquitously in eukaryotes. CK2 is composed of two subunits, the alpha catalytic and beta regulatory subunits, which form the tetrameric structure $\alpha_2\beta_2$. However, both subunits can associate independently with other proteins, probably to regulate their functions in the cell (Boldyreff and Issinger 1997; Chen et al. 1997; Hériché et al. 1997; Willert et al. 1997). In addition, the alpha subunit has been isolated as a monomer with full activity from different species, especially plants (Dobrowolska et al. 1992; Ospina et al. 1992; Klimczak and Cashmore 1994; Espunya and Martìnez 1997).

CK2 has been widely studied in several organisms, demonstrating that it is involved in different processes such as cell proliferation (Seldin and Leder 1995), cell cycle progression (Hanna et al. 1995; Espunya et al. 1999; Barz et al. 2003), signal transduction (Chen et al. 1997), and transcriptional control (Lüscher et al. 1989). The majority of the CK2 substrates — more than 300 proteins identified to date — are nuclear proteins involved in DNA/RNA-associated functions, or cellular proteins involved in signal transduction (Meggio and Pinna 2003). In particular, CK2 has been shown to phosphorylate clock proteins in *Drosophila* (Lin et al. 2002; Akten et al. 2003), *Neurospora* (Yang et al. 2002), and *Arabidopsis* (Sugano et al. 1998, 1999; Daniel et al. 2004; Portolés and Más 2007), suggesting that it is an evolutionarily conserved component of molecular clocks across several kingdoms. In *Arabidopsis*, overexpression of CKB3 and CKB4 shortened the phase of circadian clock controlled genes and reduced the days to

Received 29 August 2007. Accepted 3 December 2007. Published on the NRC Research Press Web site at genome.nrc.ca on 20 February 2008.

Corresponding Editor: G. Scoles.

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 doi:10.1139/G07-092

flowering under short and long days (CKB3) and short days (CKB4) (Sugano et al. 1999; Portolés and Más 2007). In rice (*Oryza sativa* L.), the heading date QTL *Hd6* encodes the alpha subunit of CK2 (Takahashi et al. 2001). The variant allele of *Hd6* in the *japonica* rice cultivar Nipponbare has a premature stop codon and encodes a truncated protein, while the corresponding functional allele in the *indica* rice cultivar Kasalath increases the days to heading under long-day conditions (Yamamoto et al. 2000; Takahashi et al. 2001).

The genetic control of variation in flowering time has been extensively studied in the tribe Triticeae (Poaceae). Major loci affecting photoperiod response have been mapped in barley (*Hordeum vulgare* L.). The *Ppd-H1* locus on chromosome 2HS controls flowering under long days but has no effect under short days, while *Ppd-H2* on 1HL controls flowering only under short days (Laurie et al. 1995). In addition, many QTLs controlling photoperiod response have been reported in barley. The *Ppd-H1* locus encodes a member of the CCT-domain pseudo-response regulator family, a class of genes involved in circadian clock functions (Turner et al. 2005). In addition, Szűcs et al. (2006) reported that the positional candidate for the photoperiod response effect at the *Vrn-H1* locus was a member of the photoreceptor gene family, *Phytochrome C*. To clone the positional candidate genes relative to the remaining photoperiod response QTLs, investigation of additional gene family classes is needed. In hexaploid wheat (*Triticum aestivum* L.), a single cDNA clone of the CK2 alpha subunit was isolated, and its duplication in each wheat genome was estimated by Southern blotting. A single copy was assigned to the homoeologous group 5 chromosomes, and closely linked to *Vrn-A1* on the long arm of wheat chromosome 5A (Kato et al. 2002). However, the second CK2 alpha subunit locus in wheat remains unmapped (Kato et al. 2002). In perennial ryegrass (*Lolium perenne* L.), two CK2 alpha subunit genes were mapped and one of the duplicated loci was coincident with the phenotypic variation of heading date (Shinozuka et al. 2005).

We investigated the possible role(s) of CK2 in the barley photoperiod response. The objectives of this study were to determine the nucleotide sequence of the cDNA clones encoding the CK2 alpha and beta subunits, and the genetic map positions of both of the subunit genes in barley. Based on the present data, we discuss whether barley CK2 subunit genes are positional candidates for photoperiod response QTLs.

Materials and methods

Construction and screening of the cDNA library

A cDNA library was constructed from mRNA prepared from 5-day-old barley (*H. vulgare* 'Minorimugi') seedlings using the ZAP-cDNA® Synthesis Kit (Stratagene). Two partial rice cDNA fragments encoding the CK2 alpha subunit (CK2A) (RefSeq accession no. NP_001058752) and the CK2 beta subunit (CK2B) (RefSeq acc. no. NP_001065415) were used as templates to generate each probe. Approximately 5×10^4 recombinants were screened by plaque hybridization and the hybridizing plaques were isolated. After 3 cycles of plaque purification, in vivo excisions of the pBluescript SK$^-$ vector were performed in the *Escherichia coli* K-12 strain XL1-Blue. The nucleotide sequences of the isolated cDNA clones were determined using the Thermo Sequenase Cycle Sequencing Kit (Amersham Pharmacia Biotech, UK) with M13 universal and reverse primers.

Plant materials for mapping

For the chromosome assignments of the barley CK2A and CK2B genes, we used *T. aestivum* 'Chinese Spring', *H. vulgare* subsp. *vulgare* 'Betzes', and a set of 'Chinese Spring'–'Betzes' disomic chromosome addition lines possessing each of 'Betzes' chromosomes 2H to 7H (Islam et al. 1981).

For the genetic mapping study, we screened 4 parental combinations: Steptoe and Morex, Harrington and TR-306, Igri and Franka, and Oregon Wolf Barley 95 (OWB-95) and OWB-96. Based on the parental RFLP data, 2 populations were chosen for the present mapping study. The first population was Steptoe/Morex doubled haploid lines developed by the North American Barley Genome Mapping Project (Kleinhofs et al. 1993). The second population was the OWB doubled haploid lines (Costa et al. 2001). In the present RFLP mapping, we used 60 lines of the Steptoe/Morex population and 67 lines of the OWB population.

RFLP assays

DNA was extracted from young leaves of each line using a modified CTAB method (Murray and Thompson 1980). Southern hybridization was conducted using the Gene Images labeling and detection system (Amersham Pharmacia Biotech, UK).

For the chromosome and chromosome arm assignments, RFLP assays were conducted by genomic Southern hybridization using barley CK2A and CK2B cDNAs as probe. Genomic DNA of 'Chinese Spring' and 'Betzes' and a set of wheat–barley disomic chromosome (arm) addition lines (Islam et al. 1981) was digested with *Apa*I, *Bam*HI, *Bgl*II, *Hin*dIII, *Eco*RI, *Eco*RV, *Dra*I, *Kpn*I, and *Xba*I.

For genetic mapping, polymorphisms between two combinations of the parents were detected by genomic Southern hybridization. Genomic DNA of Steptoe, Morex, OWB-95, and OWB-96 was digested with *Apa*I, *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Pst*I, *Xba*I, *Xho*I, *Alu*I, *Dde*I, *Hae*III, *Sal*I, and *Sca*I. Genotypes for each line of the mapping populations were scored.

Linkage analysis

To locate barley CK2A and CK2B genes on the known genetic maps, the genotype data sets of each polymorphism were added to the previous genotype data sets (Kleinhofs et al. 1993; Costa et al. 2001). Linkage analysis was conducted using the "near" and "try" commands of MAPMAKER/EXP 3 (Lander et al. 1987). The recombination frequencies were converted to centimorgans (cM) using the Kosambi mapping function (Kosambi 1944).

PCR–Southern blotting

To determine the original locus of the barley CK2A sequence, the following set of primers was developed for amplification of the whole putative exon sequence: Hvck2a-F (5′-ATGGCCGCATGAGCGATGC-3′) and Hvck2a-R (5′-TCATTGCGGTCGTGCCCTG-3′). Each 15 μL amplification reaction contained 0.75 U of TaKaRa Ex *Taq* polymer-

ase (TaKaRa) and 50 ng of template DNA. Amplification was performed under the following conditions: 94 °C for 1 min followed by 40 cycles of 94 °C for 1 min, 66 °C for 1 min 30 s, and 72 °C for 8 min with a final extension of 72 °C for 8 min. Amplification products were separated on 1.0% agarose gel for 0.5 h at 100 V and visualized with ethidium bromide.

Southern analysis was performed to confirm that the PCR products were homologous to the CK2A sequence. The products were separated on 1.0% agarose gel and transferred to a nylon membrane (Hybond-N+), hybridized with the barley cDNA of the CK2A gene, and detected using the Gene Images labeling and detection system (Amersham Pharmacia Biotech, UK).

Results

cDNA cloning of the CK2A and CK2B genes

Plaque hybridization of the barley cDNA library identified two positive cDNA clones encoding CK2A and two positive cDNA clones encoding CK2B. The inserts of the cDNA clones were subcloned into the pBluescript SK⁻ vector by in vivo excisions and sequenced. Figure 1A shows the entire nucleotide and deduced amino acid sequences of the longest cDNA clone encoding CK2A. The protein encoded by the ORF consists of 382 amino acids and exhibits high sequence similarity to other plant CK2As (data not shown). In addition, characteristic domains such as the ATP binding site, basic stretch (NLS), catalytic loop, and activation segment are completely conserved. Figure 1B shows the entire nucleotide and deduced amino acid sequences of the longest cDNA clone encoding CK2B. The protein encoded by the ORF consists of 259 amino acids and exhibits 73% sequence similarity with *A. thaliana* CK2B (data not shown). Characteristic domains such as the N-terminal extension region, KEN box, D-box, acidic stretch, zinc finger domain, and positive regulatory region are completely conserved. We therefore concluded that these cDNA clones encode barley CK2A (gene *HvCK2A*, GenBank acc. no. AB252049) and CK2B (gene *HvCK2B*, GenBank acc. no. AB252050).

Molecular mapping of *HvCK2A*

Chromosome assignments

For each of the 9 restriction enzymes, the Southern hybridization study identified 1 to 4 restriction fragments that hybridized with *HvCK2A* in 'Betzes'. Of these, the 10.0 kb *Bam*HI, 2.0 kb *Bgl*II, 2.1 kb *Hin*dIII, 3.5 kb and 7.5 kb *Eco*RI, 6.6 kb *Eco*RV, 3.0 kb *Dra*I, 9.5 kb *Kpn*I, and 2.5 kb *Xba*I fragments were assigned to the barley 2H chromosome (Fig. 2A). The 4.5 kb *Bam*HI, 3.5 kb *Bgl*II, 5.5 kb *Dra*I, 6.6 kb *Kpn*I, and 7.5 kb *Xba*I fragments were assigned to the barley 5H chromosome (Fig. 2A). We concluded that the barley genome has duplicate copies of the *HvCK2A* gene located on chromosomes 2H and 5H.

Genetic mapping on chromosome 2H

Among all the fragments obtained from 10 restriction enzymes, the 9.5 kb *Dra*I fragment on the 2H chromosome of 'Betzes' was detected in OWB-96 but not in OWB-95. The 18.5 kb *Dra*I fragment was specific to OWB-95. We used these two fragments for further genetic mapping. Genotype data of the doubled haploid lines show that the OWB-96-specific 9.5 kb *Dra*I fragment and the OWB-95-specific 18.5 kb *Dra*I fragment co-segregate and contribute to a single locus. Using the "near" command of MAPMAKER with the threshold of the likelihood value at 3.0, we found that 5 markers (X2.1, Pox, MWG949B, Hot1, Ebmac0684) on the short arm of chromosome 2H and 3 markers (ABG356, X2.2, and Bmac0113E) on the long arm of chromosome 2H showed linkage relationships with the genotype data of the two fragments. With the "try" command, the *HvCK2A* gene was always located in the centromeric region, between Ebmac0684 on the short arm and ABG356 on the long arm, and was designated *HvCK2a-2H* (Fig. 3A).

To determine the chromosome arm, the *HvCK2A* probe was hybridized to digested total DNA of CS + 2HL and CS + 2HS. The probe hybridized to two fragments, a 6.6 kb *Eco*RI fragment and a 9.5 kb *Kpn*I fragment. Both fragments were assigned to the short arm of chromosome 2H (data not shown). We concluded that *HvCK2a-2H* is located on the short arm of chromosome 2H in the following position: X2.1, Pox, MWG949B, Hot1, Ebmac0684, *HvCK2a-2H*, centromere, ABG356, X2.2, Bmag0113E. Moreover, *HvCK2a-2H* was found to be linked to Ebmac0684 by 2.4 cM and to ABG356 by 7.2 cM (Fig. 3A).

Genetic mapping on chromosome 5H

Using 16 restriction enzymes, 5 polymorphic fragments were identified between Steptoe and Morex. Steptoe-specific fragments were the 5.5 kb *Bam*HI and 7.0 kb *Xba*I fragments. Morex-specific fragments were the 10.0 kb *Apa*I, 9.4 kb *Bam*HI, and 9.4 kb *Xba*I fragments. In the doubled haploid lines, all polymorphic fragments co-segregated and contributed to a single locus. Using the "near" command of MAPMAKER with the threshold of the likelihood value at 3.0, we identified 26 markers linked to *HvCK2A* on the long arm of chromosome 5H. With the "try" command, the *HvCK2A* gene on chromosome 5H (designated *HvCK2a-5H*) was always located between WG644 and ABG712, 1.0 cM from each marker (Fig. 3B).

Chromosome assignment of the *HvCK2A* sequence

To determine which chromosome contains the original *HvCK2A* sequence (Fig. 1), PCR-Southern hybridization was conducted using genomic DNA of 'Chinese Spring' and 'Betzes' and a set of wheat–barley disomic addition lines. With the primer set Hvck2a-F and Hvck2a-R, a single 5.7 kb fragment was amplified from the genomic DNA of 'Chinese Spring' (Fig. 4A, lane 1) and hybridized with the *HvCK2A* probe (Fig. 4B, lane 1). In 'Betzes', a single 5.0 kb fragment was amplified (Fig. 4A, lane 2) and hybridized with *HvCK2A* (Fig. 4B, lane 2). Among the set of 'Betzes' chromosome addition lines, a 'Betzes'-specific 5.0 kb fragment was amplified and hybridized with the *HvCK2A* sequence in the 5H chromosome addition line (Fig. 4A, lane 6; Fig. 4B, lane 6). We concluded that the present cDNA sequence of *HvCK2A* originated from the genomic region encoding the CK2A gene on chromosome 5H. In comparison with the 1158 bp cDNA sequence between primers Hvck2a-F and Hvck2a-R, the genomic sequence of

Fig. 1. The nucleotide and deduced amino acid sequences of barley cDNAs encoding (A) casein kinase 2 alpha subunit (CK2A; acc. no. AB252049) and (B) casein kinase 2 beta subunit (CK2B; acc. no. AB252050). The stop codon is denoted by an asterisk. Characteristic domains of CK2A and CK2B are underlined: (A) ATP binding site, basic stretch (NLS), catalytic loop, and activation segment; (B) N-terminal extension region, KEN box and D-box (putative degradation motifs), acidic stretch, zinc finger domain (dimer formation), and positive regulatory region (binding to catalytic subunit).

(A)

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                                                    GGTTCATCTTCAGCTAATCTCTCCCTCC   28
TCCACCCACCCACCCACCAGCCGGAAACCCTAGCCCGCCCTCGCCGCCGGCGATGGCCGC   88
ATGAGCGATGCCCCTCCGAGGAAGCGCCCACCAGCCAGCTCCGTAGCTCGAGCCTCCGCC  148
M  S  D  A  P  P  R  K  R  P  P  A  S  S  V  A  R  A  S  A

GCCGCCGTCGTTGTAGCCGCCCTCGCGTCCTCCTTCATCGCCCTGTCCCCTCGCTGCACC  208
A  A  V  V  V  A  A  L  A  S  S  F  I  A  L  S  P  R  C  T

CCGGCCGCCGCGGGATCCGGGCCCAGCATGTCGAAGGCGAGGGTCTACACCGACGTCAAC  268
P  A  A  A  G  S  G  P  S  M  S  K  A  R  V  Y  T  D  V  N

GTGGTGCGCCCCAAGGAGTACTGGGACTACGAGGCGCTCACCGTCCAGTGGGGTGAGCAG  328
V  V  R  P  K  E  Y  W  D  Y  E  A  L  T  V  Q  W  G  E  Q

GATGACTATGAGGTTGTCAGGAAAGTTGGAAGAGGTAAATATAGTGAAGTGTTTGAAGGC  388
D  D  Y  E  V  V  R  K  V  G  R  G  K  Y  S  E  V  F  E  G
                                  ATP binding site
TTCAGTGTTAACAATAGCGAGAAATGTGTCATTAAGATACTCAAGCCTGTAAAGAAAAAG  448
F  S  V  N  N  S  E  K  C  V  I  K  I  L  K  P  V  K  K  K

AAGATTAAAAGGGAGATAAAAATACTTCAGAACCTCTGTGGAGGTCCAAATATCATCAAG  508
K  I  K  R  E  I  K  I  L  Q  N  L  C  G  G  P  N  I  I  K
Basic stretch (NLS)
CTGCTTGATATTGTCAGGGATCAACATTCGAAAACTCCCAGCTTGATCTTTGAATATGTC  568
L  L  D  I  V  R  D  Q  H  S  K  T  P  S  L  I  F  E  Y  V

AACAACACAGATTTTAAAGTGCTCTATCCCACGTTGACAGATTATGATATTCGCTACTAC  628
N  N  T  D  F  K  V  L  Y  P  T  L  T  D  Y  D  I  R  Y  Y

TTATACGAGCTACTAAAGGCATTAGATCACTGCCATTCACAAGGCATTATGCACCGAGAT  688
L  Y  E  L  L  K  A  L  D  H  C  H  S  Q  G  I  M  H  R  D

GTCAAGCCCCATAATGTTATGATTGATCATGATCTTCGAAAACTTCGCTTGATAGACTGG  748
V  K  P  H  N  V  M  I  D  H  D  L  R  K  L  R  L  I  D  W
Catalytic site
GGCCTGGCGGAGTTTTACCATCCAGGCAAGGAATACAATGTCCGTGTTGCTTCAAGGTAT  808
G  L  A  E  F  Y  H  P  G  K  E  Y  N  V  R  V  A  S  R  Y
                                  Activation segment
TTCAAGGGACCTGAACTTCTAGTTGATTTGCAAGATTACGATTATTCTCTGGACATGTGG  868
F  K  G  P  E  L  L  V  D  L  Q  D  Y  D  Y  S  L  D  M  W

AGCCTTGGGTGCATGTTTGCTGGGATGATCTTCCGCAAGGAGCCATTCTTCTATGGCCAT  928
S  L  G  C  M  F  A  G  M  I  F  R  K  E  P  F  F  Y  G  H

GATAACCATGACCAACTTGTGAAAATTGCGAAGGTACTTGGAACAGACAGCCTGAATGCT  988
D  N  H  D  Q  L  V  K  I  A  K  V  L  G  T  D  S  L  N  A

TACTTAAAGAAGTACCACCTTGAGCTTGACCCTCAGCTTGAACATCTTGTTGGAAGGCAC 1048
Y  L  K  K  Y  H  L  E  L  D  P  Q  L  E  H  L  V  G  R  H

AGTAGAAAACCCTGGTCAAAGTTCATTAATGCTGATAACCAGCATCTAGTATCTCCCGAG 1108
S  R  K  P  W  S  K  F  I  N  A  D  N  Q  H  L  V  S  P  E

GCCATAGATTTTCTCGATAAGCTTCTTCGCTATGATCACCAAGATAGGCTCACTGCTCGT 1168
A  I  D  F  L  D  K  L  L  R  Y  D  H  Q  D  R  L  T  A  R

GAAGCTATGGCGCATCCATACTTCCTTCAAGTGAGAGCAGCGGAGAACAGCAGGGCACGA 1228
E  A  M  A  H  P  Y  F  L  Q  V  R  A  A  E  N  S  R  A  R

CCGCAATGACATTGAGTGTGCACCGTGCAAAGTGGAGCAGTTGCTAATTGTTACTGATCA 1288
P  Q  *

TCACATGATGGAACTGTCACGATGGCAACTTGATCCAACTATCCGTCCTATGCACTGAGA 1348
TGGCGGTTTGAGTCGCTATGCTGTAAGAGCTTGTAACATTGTACTATTTGTAAACTTCAG 1408
AGCACTCTGTTTACTCCAGTGATAGTGGTCGATGAATGTGGTGGCATGCATGCCACCATT 1468
TGTGTAACAGGGTAAGTAAATTGCCTGCATTGGGCAGTCCTAAGACCCAACAGTTTACTT 1528
GTGTTGTTAAGAGTTACAATAGTCAAATGTATGGTCATTTTTTATTGCGATTATTTTGTA 1588
CTGTTTTTGAAACAAAAAAAAAA
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'Betzes' was 5.0 kb. These results demonstrated that the total intron is almost four fifths of the genomic coding region of *HvCK2a-5H*.

Fig. 1 (*concluded*).

(B)

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                                                    GCTCGTTCCTCCCCCCACCTGCGCACCAAGTTCAAACCCTA   41
GTCCAGCCTGCGAGCGCCGACGCGTCGCGCCGCGCGGCTGCCGGCAAGCAGGGGCTCCGG  101
ACCACAGCGGGGGATGAGCGGCGCAGCTACAGGGATCGGGGTTTCGGCGGCGCCGCGGAG  161
ATGGACCGGAAGCGCATCAAGGAGGCGCTGGAGAAACACACGGAAAGGCCGTCCCCGTCC  221
M  D  R  K  R  I  K  E  A  L  E  K  H  T  E  R  P  S  P  S
                                  N-terminal region
ACCTCCAGGGGGGCGTCCAGGGAGAAGGAGATGCTCGCCGCCGGCAAGATAACCACCCAG  281
T  S  R  G  A  S  R  E  K  E  M  L  A  A  G  K  I  T  T  Q

ATCGGCAAGGTCCCCAAAGTCTCCGATGTCGAGGAATTCGAAACTGACAGTGAAGATTCT  341
I  G  K  V  P  K  V  S  D  V  E  E  F  E  T  D  S  E  D  S

GATGTTAGCGGTTCTGAAGGAGAGGACACATCTTGGATTTCATGGTTCTGTAGCTTGCGA  401
D  V  S  G  S  E  G  E  D  T  S  W  I  S  W  F  C  S  L  R

GGCAACGAATTCTTCTGTGAGATTGATGATGATTATATACAGGATGATTTCAATCTCTGT  461
G  N  E  F  F  C  E  I  D  D  D  Y  I  Q  D  D  F  N  L  C

GGCCTAAGCAATCAGGTGCCATATTATGATTATGCACTTGATCTCATCCTAGACATTGAG  521
G  L  S  N  Q  V  P  Y  Y  D  Y  A  L  D  L  I  L  D  I  E
KEN box                                   D-box
TCTTCTAATGGTGATGTATTCACTGAGGAGCAAAATGAATTAATTGAGTCATCTGCAGAG  581
S  S  N  G  D  V  F  T  E  E  Q  N  E  L  I  E  S  S  A  E
                  Acidic stretch
ATGCTGTATGGTTTAATCCATGCACGGTACATCTTAACTAGCAAGGGTCTAGCTGCAATG  641
M  L  Y  G  L  I  H  A  R  Y  I  L  T  S  K  G  L  A  A  M

TTAGAAAAGTTCAAGAATTATGATTTTGGCAGATGCCCTCGAGTATACTGCTGTGGCCAG  701
L  E  K  F  K  N  Y  D  F  G  R  C  P  R  V  Y  C  C  G  Q

CCCTGTCTTCCAGCAGGGCAATCAGACATTCCTAGGTCAAGCACAGTGAAGGTGTTTTGT  761
P  C  L  P  A  G  Q  S  D  I  P  R  S  S  T  V  K  V  F  C
                                  Zinc finger domain
CCAAAATGTGAAGACTTACACTATCCAAGGTCCAAGTACCAAGGCAACATTGATGGAGCA  821
P  K  C  E  D  L  H  Y  P  R  S  K  Y  Q  G  N  I  D  G  A

TACTTTGGTACGACGTTCCCTCATCTCTTCTTGATGACATATCCACACCTGAAGCCACAG  881
Y  F  G  T  T  F  P  H  L  F  L  M  T  Y  P  H  L  K  P  Q

AAGCCATCACAGCAATACGTTCCAAGGGTTTTTGGCTTCAAACTTCACAAGCAGTCGTGA  941
K  P  S  Q  Q  Y  V  P  R  V  F  G  F  K  L  H  K  Q  S  *
                                  Positive regulatory region
CAAACTTTTACCAGCGGGCGCAATTTGTTTTTACCTAGTGTTAGAGATGGAACACCATGG 1001
CCTCAAAATGTTGTGGCCTCTTGATTCGAGTACTCGCGAGCTTGAATCTTGCTGTCAACT 1061
GCTGAGCAACTGTATCTGTTTTGTTGTATGACTGCTGTGTAATCTAGGTTTCTTCACTTG 1121
ATCAAGCTATGCACATTTGCATGTTAAAAAAAAAAAAAAAAAA                  1164
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Molecular mapping of *HvCK2B*

Chromosome assignments

One to three restriction fragments hybridizing with *HvCK2B* were detected in 'Betzes' DNA digested by 6 restriction enzymes (Fig. 2). The 15.0 kb *Apa*I, 6.6 kb *Bam*HI, 9.4 kb and 3.0 kb *Bgl*II, 5.0 kb and 2.5 kb *Dra*I, and 2.1 kb and 10 kb *Xba*I fragments were specific to 'Betzes'. No fragment was detected in 'Betzes' by *Kpn*I digestion. From Southern blotting of each chromosome addition line, no 'Betzes' fragment was assigned to chromosome 2H, 3H, 4H, 5H, 6H, or 7H. The data demonstrated the possibility that *HvCK2B* is located on chromosome 1H.

Genetic mapping

RFLP assays were conducted with 11 restriction enzymes to detect polymorphic fragments between Steptoe and Morex. Of all the fragments detected in Steptoe and Morex in the present study, only one was polymorphic between the parental lines. The 15 kb *Eco*RI fragment was present in Morex but not in Steptoe. Genotype data of this fragment were added to the previously reported mapping data. Using the "near" command of MAPMAKER with the threshold of the likelihood value at 3.0, we identified 25 markers linked to *HvCK2B* on the long arm of chromosome 1H. Using the

Fig. 2. Chromosome assignment of the casein kinase 2 alpha subunit gene (A) and beta subunit gene (B) in *Hordeum vulgare* 'Betzes'. Genomic DNA from *Triticum aestivum* 'Chinese Spring' (lane 1), 'Betzes' (lane 2), and wheat–barley disomic chromosome addition lines CS + 2H (lane 3), CS + 3H (lane 4), CS + 4H (lane 5), CS + 5H (lane 6), CS + 6H (lane 7), and CS + 7H (lane 8). The molecular weight (kilobases) is presented on the left side of each panel.

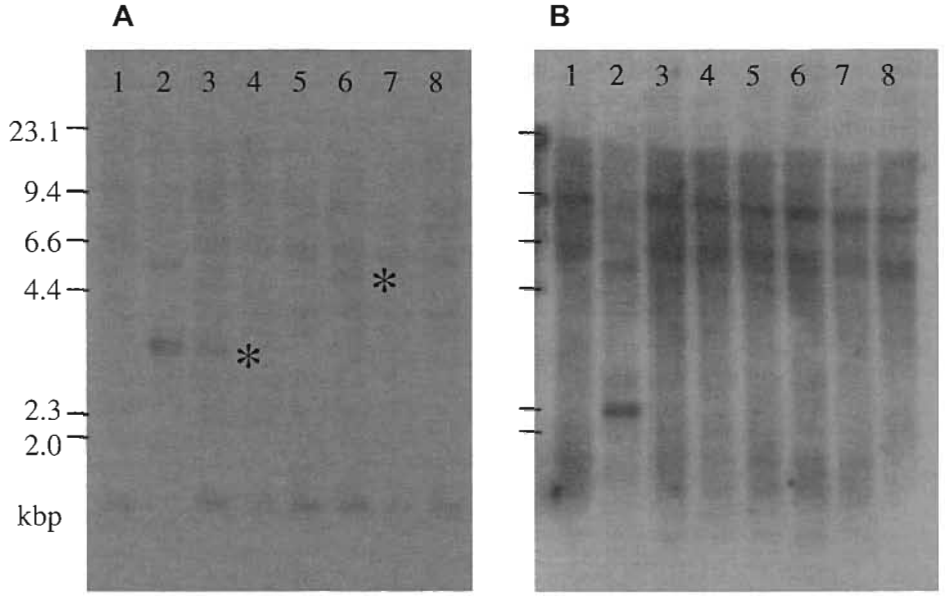
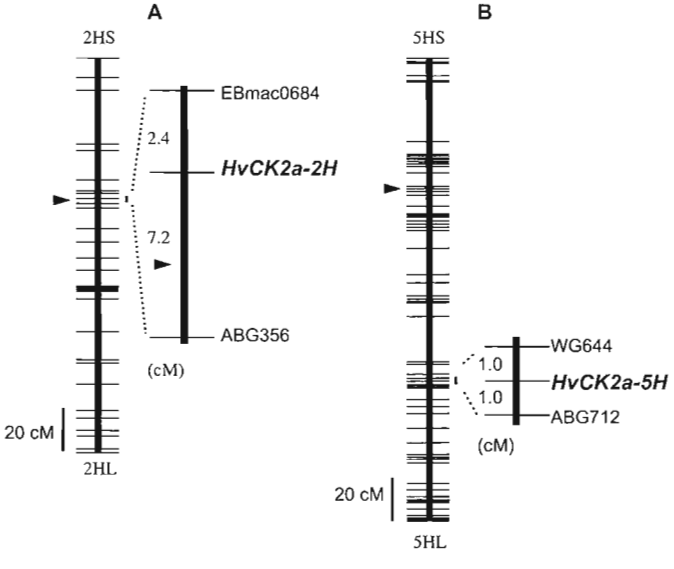


Fig. 3. Genetic linkage map of duplicate casein kinase 2 alpha subunit (CK2A) genes on chromosomes 2H and 5H in barley (*H. vulgare*). In the Oregon Wolf Barley mapping population, the CK2A gene was located between Ebmac0684 on the short arm and ABG356 on the long arm of barley chromosome 2H, and designated *HvCK2a-2H* (A). In wheat–barley 2H chromosome arm disomic addition lines, the centromere was assigned between *HvCK2a-2H* and ABG356 in the present study. In doubled haploid lines of a cross between Steptoe and Morex, the CK2A gene was located between WG644 and ABG712 on the long arm of barley chromosome 5H and designated *HvCK2a-5H* (B). Black arrows indicate the centromere positions.



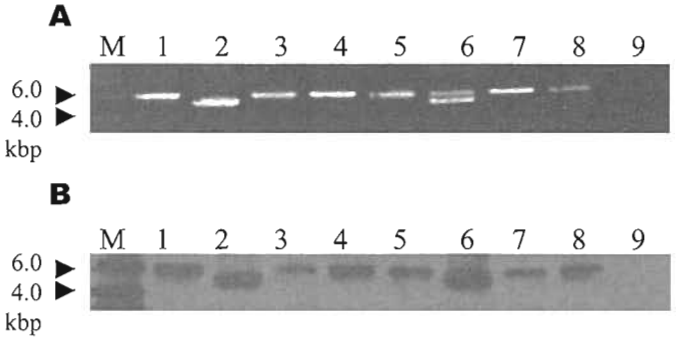
"try" command, we found that *HvCK2B* co-segregated with CDO105B and MWG800, was located between ABR337 and ABG494, and was linked to ABR337 and ABG494 by 4.0 cM and 6.0 cM, respectively (Fig. 5).

Discussion

Three CK2 subunit genes are coincident with heading time QTLs

CK2 is essential for photoperiodic regulation of flowering time, as shown by studies of CKB3 and CKB4, which are casein kinase 2 regulatory subunits, overexpressed in transgenic *Arabidopsis* plants (Sugano et al. 1999; Portolés and Más 2007). In rice, a CK2 alpha subunit encoded by *Hd6* is involved in the photoperiodic flowering response (Takahashi et al. 2001). These findings demonstrate the importance of CK2 function in the regulation of flowering in both long-day and short-day plants. The genetic control of variation in flowering time has also been extensively studied in the tribe Triticeae. The objective of the present study was to map the CK2 subunit genes and compare the positions of these genes with the reported QTL positions (GrainGenes; http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi). Conserved genomic locations for genes involved in vernalization and photoperiodic induction have been identified among species by comparative genetic studies (Dubcovsky et al. 1998). Comparative maps between barley and wheat have demonstrated that *HvCK2a-5H* should be orthologous to the wheat locus *tck2a*, which has been found to be closely linked to *Vrn-A1* on the long arm of wheat chromosome 5A (Kato et al. 2002). In barley, the marked variation of days to heading is dependent on both vernalization and day length: in doubled haploid lines derived from the cultivars Dicktoo and Morex, a heading QTL responsive to vernalization was found on chromosome 7, at a syntenic region with wheat *Vrn-A1* (Pan et al. 1994). The vernalization effect could be due to allelic variation at the *Vrn-H1* (*Sh2*) locus (Pan et al. 1994). Our data demonstrate the possibility that an alternative photoperiod response effect corresponds to the *HvCK2a-5H* gene. The Dicktoo allele is associated with an increase in the days to heading under long-day conditions when com-

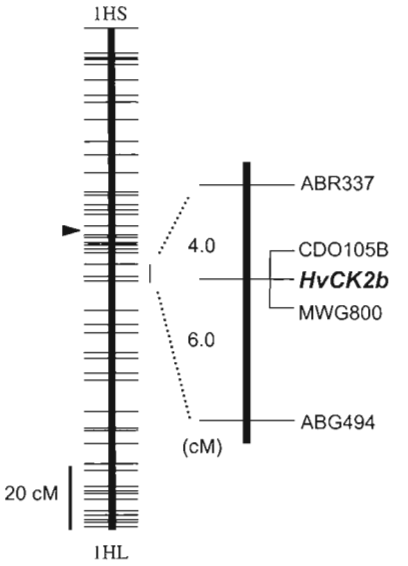
Fig. 4. PCR–Southern blotting for chromosome assignment of the *HvCK2A* sequence. (A) Electrophoresis of PCR products using the primer set Hvck2a-F (5′-ATGGCCGCATGAGCGATGC-3′) and Hvck2a-R (5′-TCATTGCGGTCGTGCCCTG-3′). Lane M, molecular size markers. PCR template was genomic DNA of *Triticum aestivum* 'Chinese Spring' (lane 1) and *Hordeum vulgare* 'Betzes' (lane 2), wheat–barley disomic chromosome addition lines CS + 2H (lane 3), CS + 3H (lane 4), CS + 4H (lane 5), CS + 5H (lane 6), CS + 6H (lane 7), CS + 7H (lane 8), and distilled water as a negative control (lane 9). (B) PCR products hybridized with the *HvCK2A* sequence.



pared with the Morex allele (Pan et al. 1994). Similarly, the functional *Hd6* allele of the *indica* rice cultivar Kasalath increased the days to heading under long-day conditions when compared with the nonfunctional allele of the *japonica* rice cultivar Nipponbare (Yamamoto et al. 2000; Takahashi et al. 2001). In addition, Szűcs et al. (2006) reported that the phytochrome gene *HvPhyC* was also located in this genomic region, showing the possibility that allelic variation of *HvPhyC* contributes to the variation in photoperiod response. The parental lines Dicktoo and Morex and the doubled haploid lines are suitable genetic materials to elucidate the functions of *HvCK2a-5H* and *HvPhyC* in photoperiod responses in barley.

Although there was no *HvCK2a-2H* transcript in the present cDNA library from 5-day-old *H. vulgare* 'Minorimugi', *HvCK2a-2H* was coincident with several heading time QTLs including *eps2*, *QHd.HaMo-2H*, *QHD.dah-2H.1*, *QHd.lgDa-2H*, *QHD.umn-2H.1*, *QHea.pil-2H.2*, and *QHea.pil-2H.3* in the centromeric region of chromosome 2H (Backes et al. 1995; Laurie et al. 1995; Marquez-Cedillo et al. 2001; Dahleen et al. 2003; Mesfin et al. 2003; Pillen et al. 2003). All QTLs were identified in field trials. To clarify whether each QTL controls the photoperiod response, the effect on heading date under variable conditions combined with photoperiod and vernalization needs to be elucidated. *HvCK2b* was located in the proximal portion of 1HL coincident with several heading date QTLs including *QHd.DiMo-1H.1* (Pan et al. 1994), *QHdHaMo-1H.1* (Marquez-Cedillo et al. 2001), and *QHD.umn-1H* (Mesfin et al. 2003). Pan et al. (1994) collected data on heading dates under variable day length with and without vernalization. *QHd.DiMo-1H.1* had a significant effect under short-day conditions but not under long-day conditions, suggesting that *QHd.DiMo-1H.1* controls photoperiod response. To investigate whether *HvCK2b* is associated with the photoperiod response at *QHd.DiMo-1H.1*, Dicktoo

Fig. 5. Genetic linkage map of the casein kinase 2 beta subunit gene (*HvCK2B*) on chromosome 1H of barley (*H. vulgare*). Using doubled haploid lines of a cross between Steptoe and Morex, *HvCK2B* co-segregated with CDO105B and MWG800 and was located between ABR337 and ABG494 on the long arm of barley chromosome 1H; it was designated *HvCK2b*. The black arrowhead indicates the centromere position.



and Morex and their doubled haploid lines are again suitable genetic materials.

Genomic organization of CK2 alpha and beta subunit genes

In animal genomes, the CK2 subunits are encoded by a maximum of 4 genes, e.g., 1 gene for the alpha subunit and 1 for the beta subunit in *Caenorhabditis elegans*, 2 genes for the alpha subunits and 1 gene for the beta subunit in human, and 2 genes for the alpha subunits and 2 genes for the beta subunits in yeast. In plant genomes, multiple genes for CK2 subunits have been reported. The *Arabidopsis* genome has 8 genes coding for CK2 subunits, 4 alpha and 4 beta subunit genes (Salinas et al. 2006), and the rice genome has at least 6 genes coding for putative CK2 subunits, 4 alpha subunit genes and 2 beta subunit genes (Rice Annotation Project Database; http://rapdb.lab.nig.ac.jp/index.html). In maize, 6 genes coding for CK2 subunits have been identified and characterized to date (Peracchia et al. 1999; Riera et al. 2001, 2003). As reported here, barley has the smallest number of genes for CK2 subunits in plants analyzed to date. Salinas et al. (2006) showed that multiple loci in *Arabidopsis* are located in the self-duplicated region, suggesting that each duplicated pair of genes could be functionally redundant. No obvious phenotype was identified in single T-DNA insertional mutant plants for all putatively duplicated CK2 subunit genes in *Arabidopsis* (Salinas et al. 2006). Barley is a good model plant for the functional analysis and regulation of CK2 activity in plants. The present study will facilitate CK2 activity regulation and functional analysis in barley.

Acknowledgements

The Steptoe/Morex doubled haploid lines were developed

by the North Barley Genome Mapping Project, and seed for the present study was kindly provided by Dr. K. Sato, Okayama University, Japan. Oregon Wolf Barley doubled haploid lines were developed and kindly provided by Oregon State University, USA. Wheat–barley disomic chromosome (arm) addition lines were kindly provided by Dr. Sasakuma, Yokohama City University, Japan; Dr. Furuta, Gifu University, Japan; and Dr. Koba, Chiba University, Japan. The authors acknowledge Nobuko Ito-Akasaka and Nanako Sumitani for their technical support.

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