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Cloning and characterization of two cDNAs encoding casein kinase II catalytic subunits in *Arabidopsis thaliana*

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Abstract

Two cDNA clones, ATCKA1 and ATCKA2, encoding casein kinase II (CKII) catalytic subunits, were cloned from *Arabidopsis thaliana* and their nucleotide sequences were determined. Both cDNAs contain 999 bp open reading frames and are 94% identical on the amino acid sequence level. The deduced amino acid sequences of ATCKA1 and ATCKA2 are very similar to that of the human CKII catalytic α subunit (72% homology). Northern blot analysis indicates that the ATCKA1 and ATCKA2 mRNAs are present in all plant organs, but that ATCKA1 transcript levels are quite low compared to those of ATCKA2. Genomic Southern blot analysis suggests that there are at least three CKII genes in the *A. thaliana* genome. We expressed the ATCKA1 and ATCKA2 cDNAs in *Escherichia coli* using a pET vector derivative and analyzed the expressed protein *in vitro*. The expressed ATCKA1 protein phosphorylated casein using either ATP or GTP. This activity was inhibited by heparin, indicating that the expressed protein has activity similar to those reported for animal and yeast CKII.

Introduction

Protein kinases have been shown to play important roles in the transduction of environmental and developmental signals in animals and yeast. Recent studies have shown that there are a variety of protein kinases in plants [1, 2, 4, 6, 13, 16, 18, 22, 33, 37]. Although it has been suggested that protein kinases have similar functions in plants, the roles of protein kinases in signal transduction remain to be elucidated in plants.

The availability of amino acid sequence data for a number of protein kinases from various organisms [17] provides a new method for the isolation and characterization of additional protein kinase family members in plants. As protein ki-

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers D10246 and D10247.

nases in eukaryotic cells have highly conserved regions [17], degenerate oligonucleotides corresponding to these regions have been used as specific hybridization probes in screening libraries to identify clones encoding protein kinases. Using this strategy, cDNA clones encoding putative protein kinases have been identified from bean, rice and maize [2, 22]. Alternatively, the polymerase chain reaction (PCR) can be used with similar oligonucleotides as primers to amplify the particular target DNA sequences between them [10, 11, 12, 24, 29]. Using the latter strategy, we have isolated several PCR-amplified DNA fragments which are different from each other and contain partial sequences of protein kinases from *Arabidopsis thaliana*. We found that one of these amplified DNA sequences has high homology with those of casein kinase II (CKII) catalytic subunits.

Casein kinase II (CKII) is a protein-serine/threonine kinase found in all cell types in animals. Its ubiquitous distribution among species and tissues implies a function central to all nucleated cells. In animals and yeast CKII activity is found both in the cytosol as well as in nuclei and substrates have been identified in both locations. Cytosolic substrates include proteins involved in translational control (eucaryotic initiation factors elF-2, -3, -4B and -5), metabolic regulation (glycogen synthase and calmodulin) and the cytoskeleton (non-muscle myosin and β-tubulin) [for review see 5, 32]. Substrates found in the nucleus include DNA topoisomerase II, RNA polymerase I and II, oncoproteins (adenovirus E1a and SV40 large T antigen) and transcription factors (SRF, Myc, Fos, Myb and CREB) [5, 32]. In contrast, there have been only a few reports of CKII in plant systems. Recently, two CKII-like enzymes, CKIIA and CKIIB, from maize seedlings have been characterized [9]. CKIIA appears to display a molecular mass of 135 kDa and have biochemical properties very similar to those of animal CKII. CKIIB, however, is a monomeric enzyme with an individual 39 KDa subunit immunologically related to the α and α' subunit of animal CKII [8, 9]. Thus it is of interest to identify CKII and characterize its function in plants. Using a PCR-amplified DNA fragment which contains a part of the CKII sequence as a probe, we have cloned two cDNAs encoding CKII catalytic subunits. Here we report the nucleotide sequences of two cDNAs encoding the CKII α subunit in *A. thaliana*. We expressed the cloned cDNAs in *Escherichia coli* cells using an expression vector and characterized the expressed protein *in vitro*.

Materials and methods

Preparation of a cDNA library and DNA templates for polymerase chain reaction (PCR)

Total RNA was isolated according to Nagy *et al.* [30] from whole plants of *A. thaliana* (Columbia ecotype) grown under continuous illumination of ca. 2500 lux at 22 °C for 4 to 5 weeks and harvested prior to bolting. Poly(A)$^+$ RNA was prepared according to Maniatis *et al.* [26]. Double-stranded cDNA was synthesized from poly(A)$^+$ RNA using Amersham's cDNA Synthesis System Plus. A library was prepared from this cDNA using Amersham's cDNA cloning system λgt11 (4×10^7 pfu/mg cDNA). The cDNA library was amplified once for the preparation of DNA templates for PCR.

Polymerase chain reaction

DNA was prepared from an amplified phage library by phenol extraction and CsCl gradient centrifugation. The DNA inserts were amplified by PCR using two oligonucleotide primers corresponding to the boundary sequences of the arms of λgt11 (5′-GGTGGCGACGACTCCTGGAGCCCG-3′ and 5′-TTGACACCAGACCAACTGGTAATG-3′). The following oligonucleotide sequences were used as primers to isolate PCR-amplified DNA fragments containing partial sequences of the CKII catalytic subunit from *A. thaliana*:

PKOID1 5′-(T/C)TIGGIAA(G/A)GGIGCITT(T/C)TCIGTIGT-3′

PKOID2 5′-(T/C)TIGGIAA(G/A)GGIGCI-TT(T/C)AG(T/C)GTIGT-3′

PKIXD 5′-A(G/A)IATIACICC(G/A)CAI-GCCCAIAT(G/A)TC-3′.

All the primers were phosphorylated with T4 polynucleotide kinase under standard reaction conditions to increase the efficiency of ligation [26]. PCR was performed using a GeneAmp kit according to the manufacturer's protocol (Perkin Elmer Cetus, Norwalk, CT, USA). Thirty-five cycles of denaturation, annealing and polymerization were carried out at 93 °C for 1 min, at 37 °C for 2 min and at 72 °C for 3 min, respectively. The amplified DNA fragments, which ranged from 400 to 600 bp in length, were cloned into the pBluescript vector pSKII$^-$ (Stratagene, La Jolla, CA, USA) digested with *Sma* I and dephosphorylated.

Cloning and sequencing of two full-length cDNAs, ATCKA1 and ATCKA2, from an A. thaliana *cDNA library*

The *A. thaliana* cDNA library (described above) was screened by plaque hybridization according to Maniatis *et al.* [26]. The 530 bp PCR product containing the conserved sequence of the CKII catalytic subunit gene family was used as a probe. The probe was labelled with ^{32}P using a random primer kit according to the manufacturer's instruction (Boehringer, Mannheim, Germany). Positive clones were plaque-purified and the DNA was isolated by phenol extraction and CsCl gradient centrifugation [26]. A 1.4 kb *Bam* HI fragment and a 1.3 kbp *Eco* RI fragment containing the ATCKA1 and ATCKA2 cDNAs, respectively, were subcloned into the pBluescript vector pSKII$^-$ (Stratagene). A DNA Sequencer Model 373A (ABI, San Jose, CA, USA) was used for DNA sequencing. Nucleotide and amino acid sequences were analyzed using the GENETYX software system (Software Development Co., Tokyo, Japan).

RNA and DNA blot hybridization analyses

Genomic Southern hybridization was performed as previously described [26]. Northern hybridization was carried out according to Maniatis *et al.* [26]. Poly(A)$^+$ RNA (0.5 μg or 5 μg) prepared from flower, root, stem, seed and leaf tissues was electrophoresed through a 1% agarose gel containing formaldehyde [26]. Nitrocellulose filters blotted with RNA were hybridized with ^{32}P-labelled DNA probes at 42 °C containing the 3′-noncoding regions of ATCKA1 or ATCKA2 in 50% formamide, 5× SSC, 25 mM sodium phosphate buffer pH 6.7, 10× Denhardt's solution and 250 μg/ml denatured salmon sperm DNA. The filters were washed in 0.1× SSC, 0.1% SDS at 65 °C [26]. A Bio Image Analyzer (Fuji Film Co., Tokyo, Japan) was used instead of autoradiography to obtain images of the filters.

Construction of pET-CKA1 and pET-CKA2 and expression of the ATCKA1 and ATCKA2 proteins in E. coli

A 1.1 kb *Bgl* II fragment containing ATCKA1 cDNA and a 1.2 kb *Bgl* II-*Bam* HI fragment containing ATCKA2 cDNA were cloned into the *Bam* HI site of pET-3C vector [36] and then transformed in *E. coli* BL21 cells. These constructs were named pET-CKA1 and pET-CKA2, respectively. Expression of the ATCKA1 and ATCKA2 proteins in *E. coli* were carried out according to the method described by Slice and Taylor [35] with some modifications. *E. coli* cells containing pET-CKA1 or pET-CKA2 were grown at 37 °C with vigorous shaking in 2YT medium (16 g Bacto tryptone, 10 g Bacto yeast extract, 5 g NaCl/l) containing ampicillin (100 μg/ml). When the culture reached a turbidity of $A_{600} = 0.8$, isopropyl-β-*D*-thiogalactopyranoside (IPTG) was added to a final concentration of 0.4 mM. The culture was divided into two portions and shaken at 24 °C or 37 °C for 4 h. The cells were harvested by centrifugation, resuspended in 60 μl of lysis buffer (30 mM MES pH 6.45, 50 mM KCl, 1 mM EDTA and 5 mM

2-mercaptoethanol) and sonicated two times for 10 s each at 0 °C, using maximum power. After centrifugation at 12000 × *g* for 15 min, the resulting pellets were resuspended in 60 μl of lysis buffer.

Detection of protein kinase activity in gels after SDS-PAGE

Protein kinase activity was detected in SDS-PAGE according to previously described methods [14], with some modifications. Aliquots (5 μl) of the cell extract were added to 5 μl of SDS sample buffer, heated at 90 °C for 2 min and subjected to SDS-PAGE as described by Laemmli [21] except that 2 mg/ml of casein was added to the running gel before polymerization. The polyacrylamide gel was washed twice to remove the SDS with 125 ml of 20% (v/v) 2-propanol and 50 mM Tris-Cl, pH 8.0 at room temperature over a period of 2 h and washed twice to remove 2-propanol at room temperature for 1 h. The gel was then completely denatured twice with 125 ml of 6 M guanidine hydrochloride, 50 mM Tris-Cl pH 8.0, 5 mM 2-mercaptoethanol at room temperature for 1 h and renatured three times with 200 ml of 0.05% Tween 40, 50 mM Tris-Cl pH 8.0, 5 mM 2-mercaptoethanol at 4 °C over a period of 18 h. The gel was then incubated at 30 °C for 1 h with gentle shaking in 25 ml of the kinase reaction buffer (40 mM Tris-Cl, 50 mM NaCl, 20 mM KCl, 10 mM $MgCl_2$, 0.1 mM EGTA, 2 mM DTT, 50 μM ATP, 2 MBq [γ-^{32}P] ATP). In some cases, GTP instead of ATP was used as a phosphate donor. The reaction was stopped by the addition of a stopping solution containing 5% TCA and 1% sodium pyrophosphate after removing the gel from the reaction mixture. The gel was washed extensively with the stopping solution then dried. A Bio Image Analyzer BAS2000 (Fuji Film Co., Tokyo, Japan) instead of autoradiography was used to obtain images of the gels and measure the intensity of the bands. The relative kinase activities were calculated based on the intensity of the bands.

Results and discussion

Isolation of a PCR-amplified DNA fragment containing a partial sequence of the CKII catalytic subunit from A. thaliana

The remarkable conservation of protein kinase genes among a wide variety of organisms allowed us to use PCR to isolate homologous sequences from *A. thaliana*. In this work two DNA sequences encoding conserved regions I and IX were used to perform PCR [17]. Degenerate oligonucleotide primers corresponding to two conserved amino acid sequences, LGKGAFSVV and DIWACGVIL, were used for PCR amplification of an *A. thaliana* cDNA template (Fig. 2). The amino acid sequence LGKGAFSVV near the N terminus is conserved in the calmodulin-dependent protein kinase II (CaMII) gene family and contains part of the motif (GXGXXGXV) that is characteristic of ATP-binding domains in other protein kinases [17]. The sequence, DIWACGVIL, is also conserved in this family and contains several residues that are also conserved among many protein kinases [17].

We obtained PCR-amplified fragments about 530 bp in length. The 530 bp PCR products were cloned into a pBluescript II vector, pSKII$^-$, at the *Sma* I site and sequenced. None of these PCR amplification products contained the motif characteristic of the CaMII family, but interestingly one of them contained a motif characteristic of the casein kinase II (CKII) catalytic subunit gene family. The deduced amino acid sequence of the 530 bp insert is 73% identical to the corresponding region of the human CKII catalytic α subunit.

Cloning and sequence analysis of two cDNA clones encoding a CKII catalytic subunit

The 530 bp PCR-amplified fragment was used as a probe in screening a cDNA library to clone cDNAs encoding CKII homologues.

Seven positive signals were obtained out of 2.5×10^5 plaques. The cDNA inserts were subcloned into pSKII$^-$ vector. Partial sequence

analysis revealed that the cloned DNA inserts had two distinct but closely related sequences. We subcloned and sequenced the largest inserts for each of these cDNAs and named them ATCKA1 and ATCKA2. Figure 1A and Fig. 1B show the nucleotide sequences and the corresponding amino acid sequences of the two cDNAs. ATCKA1 (Fig. 1A) contains a 999 bp open reading frame, encoding a polypeptide of 333 amino acids and has a predicted molecular weight of 39 211. ATCKA2 (Fig. 1B) contains a 999 bp open reading frame, encoding a polypeptide of 333 amino acids and has a predicted molecular weight of 39 311. ATCKA1 contains the polyadenylation consensus sequence, AATAAA, but ATCKA2 does not. The ATCKA1 and the ATCKA2 coding regions are 86.3% identical at the nucleotide sequence level.

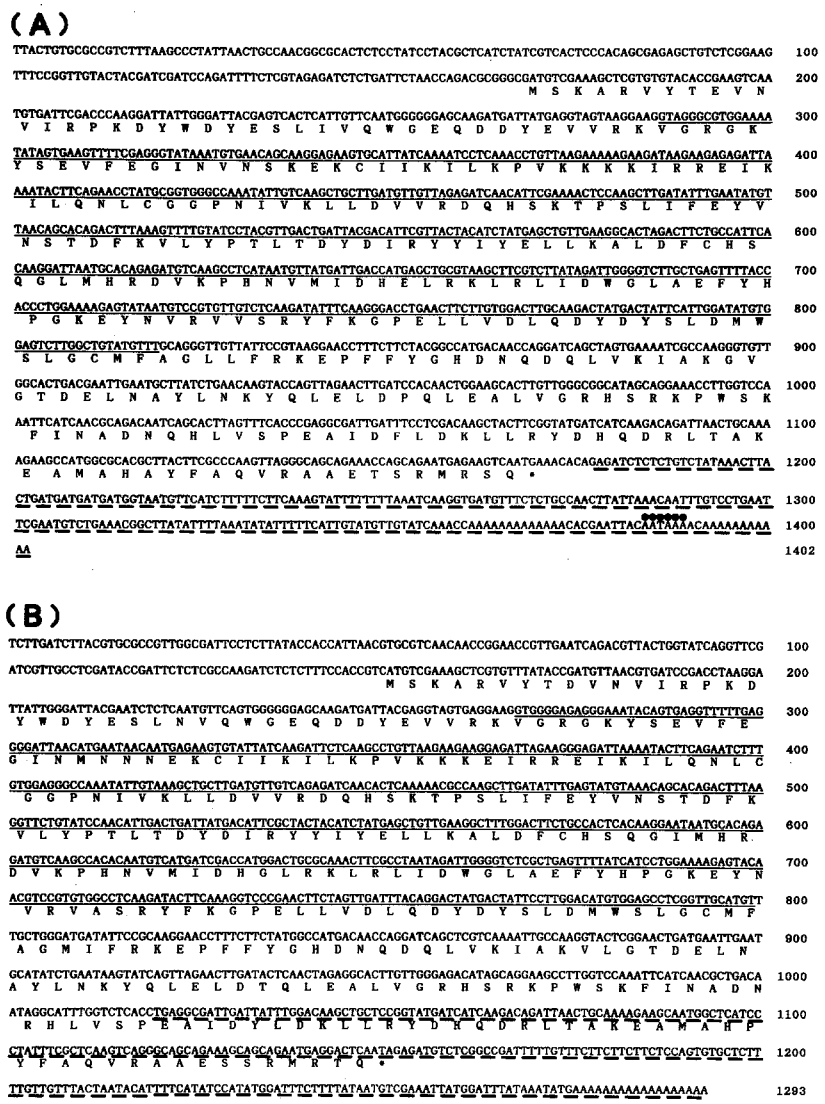

Fig. 1. DNA and deduced amino acid sequences of two cDNAs, ATCKA1 (A) and ATCKA2 (B). The DNA sequences contain the coding regions and the 5′- and 3′-noncoding regions. The amino acid sequences of the putative coding regions are shown beneath the DNA sequences. The sequences corresponding to the probe (a 530 bp PCR-amplified sequence) used to screen the library are underlined. The dashed lines indicate the sequences corresponding to the gene-specific probes used for northern blot analysis. Closed circles indicate the conserved polyadenylation sequence (AATAAA).

Primary structure of the putative ATCKA1 and ATCKA2 CKII proteins

Animal and yeast CKII proteins consist of two subunits, α and β, with molecular mass ranges of 37–44 kDa and 24–28 kDa, respectively, and an apparent subunit composition of $\alpha_2\beta_2$ [5]. The α subunit is known to be catalytic as demonstrated by expression of its gene in *E. coli* [23]. There is a second form of the α subunit, designated α′, but the function of this subunit is not yet clear [5]. The role of the β subunit appears to be that of a regulatory element, as it is phosphorylated by exogenous kinases and contains sites of autophosphorylation [5].

The amino acid sequences of the putative catalytic domains of ATCKA1 and ATCKA2 are compared with those of other CKII catalytic subunits in Fig. 2A. ATCKA1 and ATCKA2 are 94.3% identical at the amino acid level. The proteins encoded by ATCKA1 and ATCKA2 share extensive sequence homology with other CKII catalytic subunits. Among these sequences of animal and yeast, ATCKA1 is most closely related to the human CKII α subunit [28] (72.0% homology), followed by the *Drosophila* CKII α subunit [34] (71.1%), the human CKII α′ subunit [25] (70.1%), the yeast CKII α′ subunit [31] (59.3%) and the yeast CKII α subunit [3] (54.4%). ATCKA2 is most closely related to the *Drosophila* CKII α subunit (73.5% homology), followed by the human CKII α subunit (73.3%), the human CKII α′ subunit (71.2%), the yeast CKII α′ subunit (60.6%) and the yeast CKII α subunit (55.2%). ATCKA1 and ATCKA2 have the highest similarities (90.1 and 91.0% respectively) to the CKII α subunit from maize, the sequence of which was recently published [7].

All of the highly conserved regions which are present in the CKII catalytic subunit sequences reported to date are also present in ATCKA1 and ATCKA2. The triangles in Fig. 2A indicate motifs characteristic of protein kinase catalytic subunits [17]. Two residues in these motifs are characteristic of CKII subunits and are different from the corresponding residues in other protein kinases, which are essentially invariant. In posi-

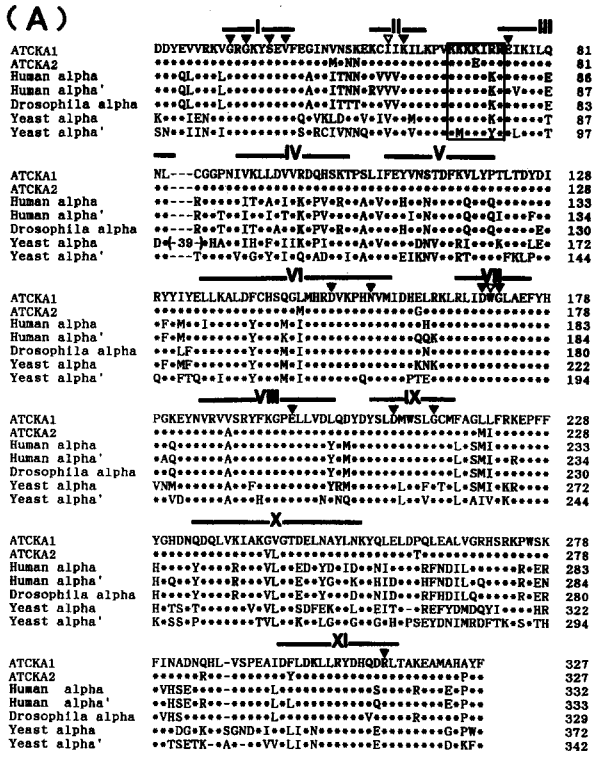

Fig. 2. A. Comparison of the deduced amino acid sequences of the catalytic domains of ATCKA1, ATCKA2, the maize CKII α subunit, the human CKII α subunit, the human CKII α′ subunit, the *Drosophila* CKII α subunit, the yeast CKII α subunit and the yeast CKII α′ subunit. Asterisks represent identical amino acid residues and dashes indicate gaps introduced to maximize alignment. In the case of the yeast CKII α subunit, an insert of 39 amino acids long was excluded from the alignment to shorten the figure. The position and length of the excluded insert within the alignment is indicated by a number within parentheses. Roman numerals indicate the eleven major conserved subdomains of protein kinases denoted by Hanks *et al.* [17]. Amino acid residues that are invariant or nearly invariant among known protein kinases are indicated by triangles. B. Comparison of the deduced amino acid sequences of the N terminus of ATCKA1, ATCKA2, the maize CKII α subunit, the human CKII α subunit, the human CKII α′ subunit, the *Drosophila* CKII α subunit, the yeast CKII α subunit and the yeast CKII α′ subunit. The boxed regions indicate conserved residues in the N termini of all of the CKII catalytic subunits sequenced to date. These include two sequence elements, SXARVY and D/EYWDYE [31].

tion 64 of CKII (numbering according to the *Drosophila* α subunit) [34] isoleucine (I^{64}) or valine (V^{64}) are present in place of alanine which is present at this position in all other protein kinases except KIN2 (which contains valine instead of alanine) and HSVK (isoleucine instead of alanine). In CKII subunits tryptophane occupies position 174 (W^{174}) in place of phenylalanine, which is present in this position in all other protein kinases. This tryptophane residue is currently believed to be unique to CKII [3, 17]. The motif D-W^{174}-G is believed to form part of the catalytic site of casein kinases. These residue variations may contribute to the unusual ability of CKII to utilize GTP in addition to ATP as a phosphate donor [5, 31, 32]. The boxed area indicates a highly basic region (KKKKIRR) located just to the C terminal side of the essential catalytic lysine (residue K^{66} in the *Drosophila* sequence). This region has been postulated to form part of the binding site for protein substrates [3].

The boxed areas in Fig. 2B show residues that are conserved among all of the N termini of the CKII catalytic subunits which have been sequenced. Of particular interest are two short blocks of sequence identity, SXARVY and (D/E)YWDYE, which lie near the N terminus. Since these two sequence elements lie outside of the catalytic domain, the fact that they are conserved presumably reflects some other critical function, perhaps interaction with the β subunit [31].

Northern and Southern blot analysis of the ATCKA1 and ATCKA2 genes

The existence of two closely related but structurally distinct isozymes of CKII catalytic subunits suggests the possibility that these isozymes may serve different functions. One reflection of functional specialization might be tissue specific expression of the two catalytic subunits. For example, the two chicken catalytic subunits, α and α′, each have tissue-specific pattern of expression [27]. We examined the relative levels of ATCKA1 and ATCKA2 transcripts in a variety of organs of *A. thaliana*. Northern blot analysis revealed the presence of ATCKA1 and ATCKA2 transcripts in all tissues examined although their expression levels varied in different tissues and with respect to each other (Fig. 3). We used 0.5 μg and 5 μg of poly(A)$^+$ RNA in the northern blot to detect ATCKA2 and ATCKA1 mRNAs, respectively. ATCKA2 transcript levels were high in root, leaf and flower tissues but quite low in seeds. ATCKA1 transcript levels were quite low (about one tenth) in all the tissues compared with those of ATCKA2. Single transcripts of 1.6 kb were detected in *A. thaliana* using each of these cDNAs as probes, although multiple transcripts have been reported for the human and chicken CKII catalytic subunits [27, 28].

The number of genes encoding the CKII catalytic subunit in the *A. thaliana* genome, was estimated by Southern blot analyses (Fig. 4). *A. thaliana* nuclear DNA was digested with *Bam* HI, *Eco* RI, *Hin*d III, *Xba* I and *Pst* I, blotted onto nylon membranes and hybridized under both high and low stringency conditions using the ATCKA1 and ATCKA2 cDNAs as probes. Under high stringency hybridization conditions ATCKA1 hybridized with one *Bam* HI, two *Eco* RI,

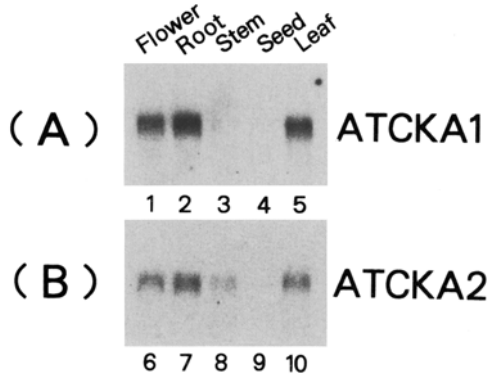

Fig. 3. Northern analysis of ATCKA1 and ATCKA2 gene expression in a variety of organs of *A. thaliana*. To detect ATCKA2 and ATCKA1 mRNA, 0.5 μg and 5 μg of Poly(A)$^+$ RNA, respectively, prepared from flower (lanes 1 and 6), root (lanes 2 and 7), stem (lanes 3 and 8), seed (lanes 4 and 9) and leaf (lanes 5 and 10) tissues was electrophoresed through 1% agarose gels containing formaldehyde [26]. Nitrocellulose filters blotted with RNA were hybridized with ^{32}P-labelled DNA fragments containing the 3′noncoding regions of either ATCKA1 or ATCKA2 as described in Fig. 1.

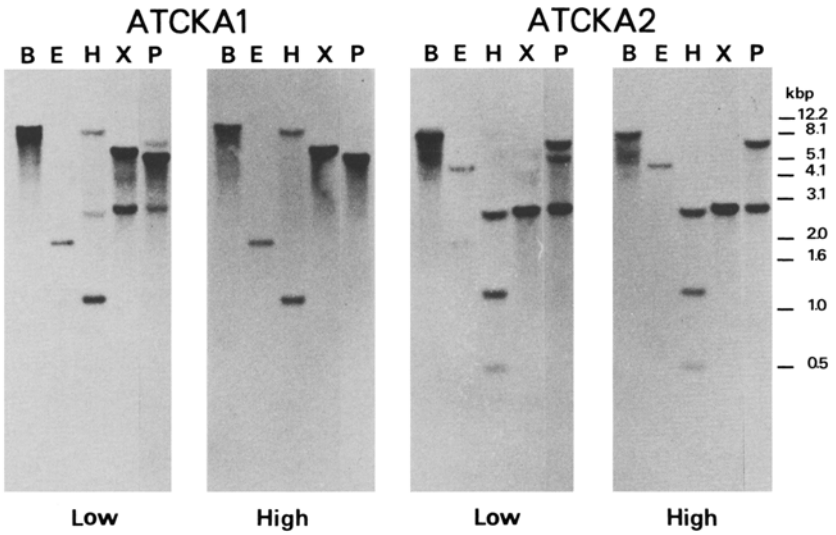

Fig. 4. Southern blot analysis of ATCKA1 and ATCKA2 genomic sequences. Genomic DNA was digested with *Bam* HI (B), *Eco* RI (E), *Hin*d III (H), *Xba* I (X) and *Pst* I (P), fractionated through 0.7% agarose gels and transferred to nylon membranes. Filters were hybridized with ^{32}P-labelled DNA fragments of ATCKA1 and ATCKA2 cDNA at 42 °C and washed in either 0.5 × SSC/0.5% SDS at 50 °C (low stringency) or 0.1 × SSC/0.1% SDS at 65 °C (high stringency). 'ATCKA1' and 'ATCKA2' indicate the probe DNA and 'High' and 'Low' represent high- and low-stringency hybridization conditions, respectively. The sizes of DNA molecular weight markers are indicated in kbp.

three *Hin*d III, one *Xba* I and one *Pst* I fragments. ATCKA2 hybridized with one *Bam* HI, one *Eco* RI, three *Hin*d III, one *Xba* I and two *Pst* I under the same conditions. However, under low-stringency hybridization conditions, other cross-hybridizing bands were detected. We observed a faint band of a 3.5 kb *Hin*d III fragment, which could not be detected in high-stringency hybridization, indicating there may be a third CKII gene in the *A. thaliana* genome.

Detection of the casein kinase activity expressed from ATCKA1 in Escherichia coli *cells*

Among a variety of protein kinases, CKII is characteristic of its ability to utilize GTP as well as ATP as a phosphate donor [5, 32]. This enzyme is inhibited by polyanionic compounds, such as heparin [5, 32]. To examine whether ATCKA1 and ATCKA2 encode CKII catalytic subunits, ATCKA1 and ATCKA2 cDNAs were expressed in *E. coli* as products of fusions with gene 10 of bacteriophage T7 using the T7 expression vector, pET-3C. BL21 cells containing the expression constructs pET-CKA1 or pET-CKA2 (Materials and methods) were grown at 37 °C and treated with IPTG at 24 °C or at 37 °C for 4 h to induce the production of the fusion proteins. ATCKA1 and ATCKA2 fusion proteins of 40 kDa were produced by IPTG treatment at 24 °C (Fig. 5). Large amounts of the ATCKA1 fusion protein were produced at 37 °C and a portion of the protein was solubilized. However, the ATCKA2 fusion protein was not solubilized in the same condition (data not shown).

In order to examine whether the ATCKA1 fusion proteins expressed in *E. coli* had CKII activity, the protein kinase activities of the proteins were measured in polyacrylamide gels containing the substrate, casein, according to Geahlen *et al.* [14]. We detected two strongly phosphorylated bands of 40 kDa and 14 kDa when the proteins from *E. coli* carrying pET-CKA1 were analyzed (Fig. 6). However, only a single band of 14 kDa was produced by proteins from *E. coli* cells carrying the pET-3C vector. Only a faint band of 40 kDa was detected when the activity was mea-

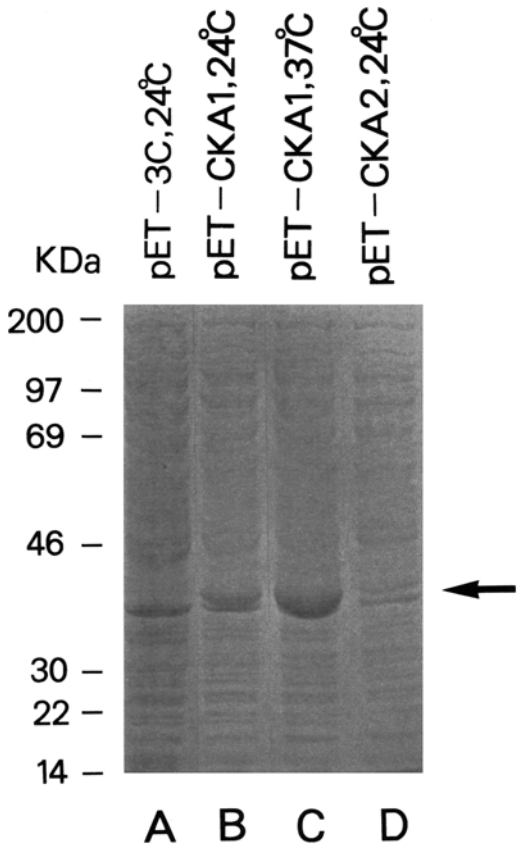

Fig. 5. SDS-PAGE analysis of the expressed proteins in *E. coli* cells containing pET-CKA1 and pET-CKA2. *E. coli* cells containing pET-3C (A), pET-CKA1 (B and C) and pET-CKA2 (D) were treated with IPTG at 24 °C or 37 °C for 4 h to induce the production of proteins encoded by these constructs. The cell extracts were analyzed by SDS-PAGE according to a standard protocol [21].

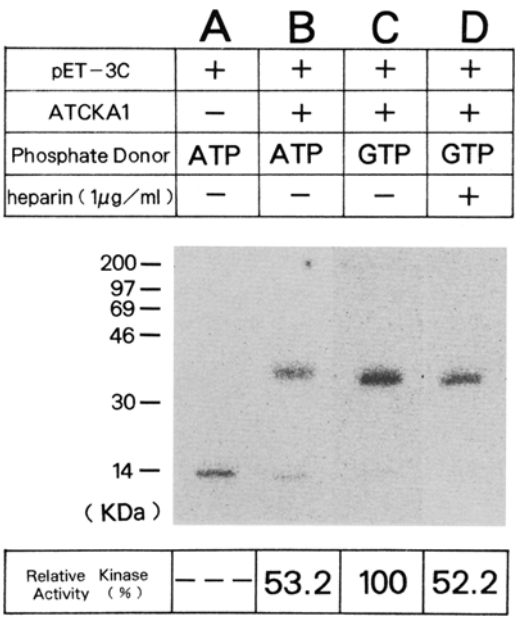

Fig. 6. Detection of the protein kinase activity of the ATCKA1 fusion product in polyacrylamide gels containing casein after SDS-PAGE. *E. coli* cell extracts were prepared as described in Materials and methods. Aliquots (5 μl) of the cell extract were electrophoresed in SDS-polyacrylamide gels containing casein (2 mg/ml). After renaturation of proteins in the gels, the protein kinase activities were determined as described in Materials and methods using extracts prepared from *E. coli* cells containing (A) the pET-3C vector and γ-^{32}P ATP as a phosphate donor, (B) the pET-CKA1 construct and γ-^{32}ATP as a phosphate donor, (C) γ-^{32}GTP as a phosphate donor or (D) γ-^{32}P GTP as a phosphate donor and heparin (1 μg/ml). The percentage of protein kinase activity in each lane is indicated at the bottom relative to the activity in lane C which is arbitrarily indicated to be 100%.

sured in a control gel in which casein was not present (data not shown). These observations suggest that the expressed protein of 40 kDa phosphorylates casein with ATP.

We then examined whether the protein can use GTP as well as ATP as a phosphate donor to phosphorylate casein. Figure 6 shows that the expressed protein of 40 kDa uses GTP as well as ATP as a phosphate donor, while the protein kinase of 14 kDa does not. In addition to this, higher phosphorylation activity was detected when GTP was used as a phosphate donor. The protein kinase activity was reduced to half by the addition of 1 μg/ml of heparin (Fig. 6), and was completely inhibited by 5 μg/ml heparin (unpublished observation). These observations indicate that the ATCKA1 fusion protein expressed in *E. coli* has protein kinase activity characteristic of the CKII catalytic subunits which have been reported in animals and yeast [5, 23].

Recently, Klimczak *et al.* [20] reported that the G-box binding factor from *A. thaliana* (GBF1) is phosphorylated by CKII-like protein kinase from broccoli. Phosphorylation of the GBF1 results in stimulation of the G-box ((A/C)(G/C)ACGTGG) [15] binding activity and formation of protein-DNA complex. The ATCKA1 and ATCKA2 CKII catalytic subunits are candidates of the

CKII components which function in phosphorylation of the GBF1 in *A. thaliana*.

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