

GENE 06925

PCR cloning and sequence of two cDNAs encoding the alpha and beta subunits of rabbit casein kinase-II

(Serine/threonine kinase; signal transduction; vascular smooth muscle cell; vascular disease)

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SUMMARY

Polymerase chain reaction (PCR) amplification of total RNA from vascular smooth muscle cells (VSMC) was used to clone and sequence full-length cDNAs encoding the α and β subunits of rabbit casein kinase-II (CK-II). A strong homology and evolutionary conservation was found in both the nucleotide (nt) and deduced amino acid sequences of CK-II from various species, with up to 100% identity among vertebrate homologues and 88% and 64% identity on the nt level with *Drosophila melanogaster* and yeast, respectively, compared with rabbit CK-II. The cloning and expression of rabbit CK-II will allow us to generate antibody and cDNA probes to investigate the role of CK-II in VSMC growth, migration, and phenotypic transformation during the pathogenesis of vascular disease.

INTRODUCTION

Casein kinase-II (CK-II) is a Ser/Thr protein kinase that has been implicated in signal transduction pathways leading to growth and differentiation. CK-II activity is modulated during the traverse of cells through the cell cycle (Carroll and Marshak, 1989; Yu et al., 1991) and in response to growth factors (Sommercorn et al., 1987). CK-II phosphorylates Ser/Thr within proteins involved in cell growth and differentiation, including proto-oncogenes C-myc (Luscher et al., 1989), C-myb (Luscher et al., 1990), and suppressor p53 (Meek et al., 1990). The cellular regulation of CK-II activity appears unique.

Unlike the classical protein kinases A or C, the cellular activity of CK-II is independent of regulation by the second messenger cAMP or Ca^{2+} (Edelman et al., 1987). The CK-II holo-enzyme is a heterotetrameric molecule composed of two catalytic, α (and/or α') and two regulatory, β subunits (Edelman et al., 1987). Recent sequencing of the enzyme from various evolutionarily disparate species like yeast (Chen-Wu et al., 1988), *Drosophila melanogaster* (Saxena et al., 1987), *Caenorhabditis elegans* (Hu and Rubin, 1990a), chicken (Maridor et al., 1991), and human (Meisner et al., 1989; Heller-Harrison et al., 1989) has revealed a high level of evolutionary conservation at both the nt and aa levels, suggesting that CK-II performs critical functions in eukaryotic organisms.

Proliferation and phenotypic transformation (a process that appears analogous to differentiation/differentiation) of VSMC plays an important role in the pathogenesis of atherosclerosis (Ross, 1986) and restenosis following PTCA (Austin et al., 1985). Our initial studies have suggested that CK-II may play a role in rabbit VSMC growth (Rothfuss et al., 1991). The rabbit is a species widely used as an experimental model for atherosclerosis and VSMC proliferative restenosis

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Abbreviations: aa, amino acid(s); bp, base pair(s); cAMP, cyclic adenosine 3',5'-monophosphate; cDNA, DNA complementary to RNA; CK-II, casein kinase-II; CK-II, cDNA (gene) encoding CK-II; kb, kilobase(s); nt, nucleotide(s); oligo, oligodeoxyribonucleotide; PCR, polymerase chain reaction; PTCA, percutaneous transluminal coronary angioplasty; SDS, sodium dodecyl sulfate; VSMC, vascular smooth muscle cells.

following vascular injury. Therefore, we have cloned and sequenced the full-length cDNA for the α and β subunits of rabbit *CK-II* for comparison of species-specific differences in aa and nt sequences. The results show that a high degree of homology exists among eukaryotic species. The cDNA probes will be suitable for studying *CK-II* expression in response to vascular injury and atherogenesis in experimental animal models and humans.

EXPERIMENTAL AND DISCUSSION

(a) PCR cloning and agarose gel analysis of RNA-PCR products

Total RNA was extracted from 10^7 rabbit aortae VSMC by the procedure of Chomczynski and Sacchi (1987). Based on the published sequences of eukaryotic α and β *CK-II* cDNAs, two pairs of consensus 20-nt oligos were synthesized. PCR amplification of the total RNA was performed with the GeneAmp RNA PCR kit (Perkin Elmer, Norwalk, CT) according to the previously published procedure (Kawasaki, 1990). The results are shown in Fig. 1. RNA (2 μ g) was reverse transcribed in two separate reactions with the downstream antisense oligos α SR and β 788R (Fig. 2, underlining arrows), specific for the α and β subunits, respectively, of *CK-II*. The upstream oligos α MF and β MF (overlining arrows) were added directly to the reaction tubes along with the PCR reaction mix and subjected to 35 cycles of amplification. Each cycle consisted of 1 min denaturation at 95°C and 1 min

of an annealing-extension step at 60°C. The final extension cycle lasted 7 min at 72°C. The PCR products were analyzed on a 1.5% agarose gel (Fig. 1A), and DNA bands corresponding to the expected sizes of the α and β subunits of *CK-II* cDNAs were extracted with the GeneClean kit (Bio101, La Jolla, CA) and subcloned into pUC18. Mini-prep plasmid DNA was made from overnight cultures of the positive colonies by the alkaline SDS lysis procedure (Ish-Horowitz and Burke, 1981) and further analyzed by restriction analysis (Fig. 1B).

(b) Sequencing and analysis of *CK-II* subunit cDNAs

The cDNA inserts were sequenced with a series of overlapping primers by using a modification of the dideoxy chain-termination method (Sanger et al., 1977) on an Applied Biosystems 373 Automated DNA Sequencer. The complete nt and deduced aa sequences of the subunit cDNAs are shown in Fig. 2. A comparison of the sequences from rabbit *CK-II* with that of the other eukaryotic species (Table I) was performed using the FASTA and TFASTA programs of GCG (Madison, WI) with gaps introduced to maximize alignment.

(c) Conclusions

PCR amplification of RNA from VSMC was used to clone full-length cDNAs encoding both the α and β subunits of rabbit *CK-II*. The deduced aa sequence reveals

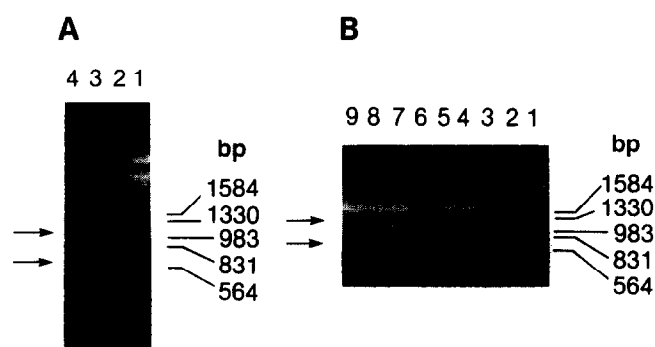


Fig. 1. Analysis of cDNA products from RNA-PCR. Numerals on right margins refer to *EcoRI* + *HindIII*-digested phage λ DNA markers in lanes 1. (Panel A) 1.5% agarose gel analysis of PCR products. DNA bands in lanes 3 and 4 (arrows) contain the full-length cDNA for the α and β subunits, respectively, of *CK-II*. The PCR product in lane 2 amplified by using the oligo pair for the β subunit had an uncharacteristic size and was not analyzed further. The cDNAs were extracted from the agarose gels and subcloned into a pUC18 cloning vector. (Panel B) *EcoRI* + *HindIII* digest of full-length cDNA products from the pUC18 subclones. Lanes 2–5 and 7 contain cDNA for the α subunit, while lanes 8 and 9 have the β subunit cDNA (see arrows). The cDNA product in lane 6 had an uncharacteristic size and was not analyzed further.

TABLE I

Comparison of nt and deduced aa sequences of *CK-II* subunits from eukaryotic species^a

Species ^b	Percent homology with rabbit sequence ^c			
	α <i>CK-II</i>		β <i>CK-II</i>	
	nt	aa	nt	aa
Rabbit	(100)	(100)	(100)	(100)
Human	95	99	95	100
Bovine	94	99	–	100
Porcine	–	–	94	100
Rat	–	97	–	–
Mouse	–	–	93	100
Chicken	86	97	84	100
<i>X. laevis</i>	81	95	80	99
<i>Drosophila</i>	74	88	77	89
<i>C. elegans</i>	70	78	67	54
<i>Z. mays</i>	70	70	–	–
<i>T. parva</i>	66	68	–	–
Yeast	60	64	–	–

^aTable compiled with sequence data obtained from GenBank.

^b*X.*, *Xenopus*; *C.*, *Caenorhabditis*; *Z.*, *Zea*; and *T.*, *Theileria*.

^ccDNA and derived aa sequence. Symbol – = not available.

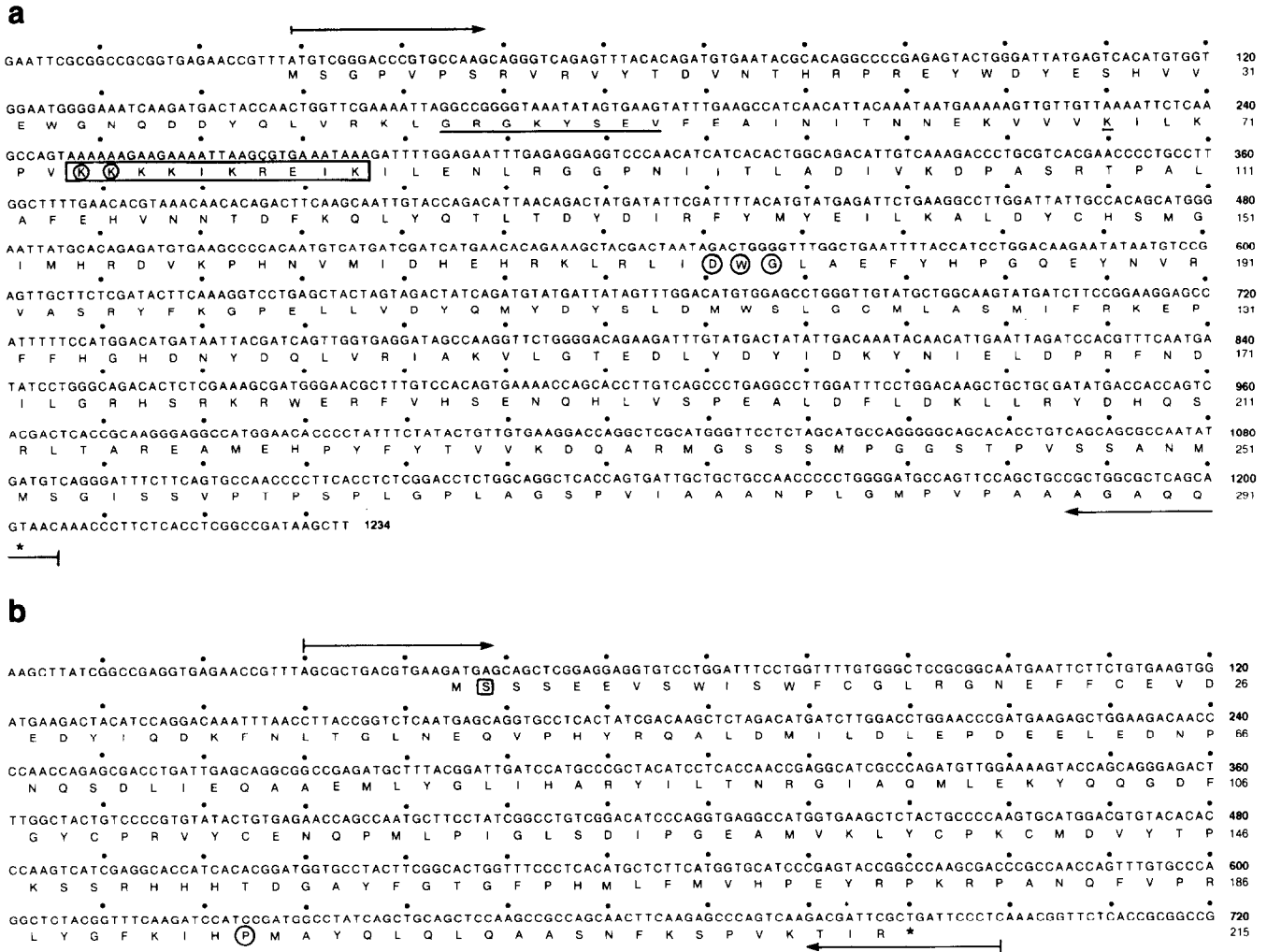


Fig. 2. The nt sequence of *CK-II* and deduced aa sequences of α and β subunits of rabbit *CK-II*. The stop codons in the open reading frames are marked by stars. (a) cDNA sequence that encodes the 391-aa α subunit protein. The sequences of the consensus PCR oligo pair α MF and α SR are marked by arrows. α SR was synthesized as the reverse complement of the indicated sequence. Motifs characteristic of the protein kinase catalytic subunits (Hanks et al., 1988) are shown. These include the underlined residues G-X-G-X-X-S-X-V and an invariant K involved in ATP binding, as well as the motif D-W-G (circled) forming part of the catalytic site of the kinase. W¹⁷⁶ is characteristic of *CK-II* catalytic subunits. The boxed aa show the putative heparin-binding domain of α subunit of *CK-II*. Mutations at K⁷⁴ and K⁷⁵ (boxed-circled) altered the heparin-binding properties of *CK-II* (Hu and Rubin, 1990b) from *C. elegans*. (b) cDNA sequence for the 215-aa β subunit protein. The sequences of the consensus PCR oligo pair: β MF and β 788R are marked by arrows. β 788R was synthesized as the reverse complement of the indicated sequence. A putative autophosphorylation site, Ser² (Maridor et al., 1991) is boxed. Heller-Harrison et al. (1989) have reported an A¹⁹⁴ instead of the conserved P¹⁹⁴ (circled).

a protein of 391 aa for the α subunit and of 215 aa for the β subunit of *CK-II*.

Comparison with sequences from other eukaryotic species shows a remarkable evolutionary conservation at both the nt and aa sequence levels.

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REFERENCES

- Austin, G.E., Ratliff, N.B., Hollman, J., Tabei, S. and Phillips, D.F.: Intimal proliferation of smooth muscle cells as an explanation for recurrent coronary artery stenosis after percutaneous transluminal angioplasty. *J. Am. Coll. Cardiol.* 6 (1985) 369–375.
- Carroll, D. and Marshak, D.R.: Serum-stimulated cell growth causes oscillations in casein kinase-II activity. *J. Biol. Chem.* 264 (1989) 7345–7348.
- Chen-Wu, J.L.-P., Padmanabhan, R. and Glover, C.V.C.: Isolation, sequencing, and disruption of the *CKA1* gene encoding the alpha subunit of yeast casein kinase-II. *Mol. Cell. Biol.* 8 (1988) 4981–4990.
- Chomczynski, P. and Sacchi, N.: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162 (1987) 156–159.

- Edelman, A.M., Blumenthal, D.A. and Krebs, E.G.: Protein serine/threonine kinases. *Annu. Rev. Biochem.* 56 (1987) 567–613.
- Hanks, S., Quinn, A. and Hunter, T.: The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* 241 (1988) 42–52.
- Heller-Harrison, R.A., Meisner, H. and Czech, M.P.: Cloning and characterization of a cDNA encoding the β subunit of human casein kinase-II. *Biochemistry* 28 (1989) 9053–9058.
- Hu, E. and Rubin, C.S.: Casein kinase-II from *Caenorhabditis elegans*. Properties and developmental regulation of the enzyme, cloning and sequence analyses of cDNA and the gene for the catalytic subunit. *J. Biol. Chem.* 265 (1990a) 5072–5080.
- Hu, E. and Rubin, C.S.: Expression of wild-type and mutated forms of the catalytic α subunit of *Caenorhabditis elegans* casein kinase-II in *E. coli*. *J. Biol. Chem.* 265 (1990b) 20609–20613.
- Ish-Horowicz, D. and Burke, J.F.: Rapid and efficient cosmid cloning. *Nucleic Acids Res.* 13 (1981) 2989–2998.
- Kawasaki, E.S.: Amplification of RNA. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J. (Eds.), *PCR Protocols*. Academic Press, San Diego, 1990, pp. 21–27.
- Luscher, B., Kuenzel, E.A., Krebs, E.G. and Eisenman, R.N.: Myc oncoproteins are phosphorylated by casein kinase-II. *EMBO J.* 8 (1989) 1111–1119.
- Luscher, B., Christenson, E., Litchfield, D.W., Krebs, E.G. and Eisenman, R.N.: Myb DNA binding inhibited by phosphorylation at a site deleted during oncogenic activation. *Nature* 344 (1990) 517–522.
- Maridor, G., Park, W., Krek, W. and Nigg, E.A.: Casein kinase II. cDNA sequences, developmental expression, and tissue distribution of mRNAs for α , α' and β subunits of the chicken enzyme. *J. Biol. Chem.* 266 (1991) 2362–2368.
- Meek, D.W., Simon, S., Kikkawa, U. and Eckhart, W.: The P53 tumor suppressor protein is phosphorylated at serine 389 by casein kinase-II. *EMBO J.* 9 (1990) 3253–3260.
- Meisner, H., Heller-Harrison, R., Buxton, J. and Czech, M.P.: Molecular cloning of the human casein kinase-II subunit. *Biochemistry* 28 (1989) 4072–4076.
- Ross, R.: The pathogenesis of atherosclerosis. *New Engl. J. Med.* 314 (1986) 448–500.
- Rothfuss, K.J., Gupta, S.K. and Singh, J.P.: Stimulation of casein kinase-II (CK-II) in vascular smooth muscle cells by PDGF. Potential role of CK-II in signal transduction leading to cell proliferation. *J. Cell Biol.* 115 (1991) 430a.
- Sanger, F., Nicklen, S. and Coulson, A.R.: DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74 (1977) 5463–5467.
- Saxena, A., Padmanabhan, R. and Glover, C.V.C.: Isolation and sequencing of cDNA clones encoding alpha and beta subunits of *Drosophila melanogaster* casein kinase-II. *Mol. Cell. Biol.* 7 (1987) 3409–3417.
- Sommercorn, J., Mulligan, J., Lozeman, F. and Krebs, E.: Activation of casein kinase-II in response to insulin and to epidermal growth factor. *Proc. Natl. Acad. Sci. USA* 84 (1987) 8834–8838.
- Yu, I.J., Spector, D.L., Bae, Y.-S. and Marshak, D.R.: Immunocytochemical localization of casein kinase-II during interphase and mitosis. *J. Cell Biol.* 114 (1991) 1217–1232.