The cDNAs coding for the $\alpha$- and $\beta$-subunits of *Xenopus laevis* casein kinase II*

Ana Jedlicki, Maria Victoria Hinrichs, Catherine C. Allende and Jorge E. Allende

Departamento de Bioquímica, Facultad de Medicina and Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 70086, Santiago 7, Chile

Received 4 December 1991

Using a Agt10 cDNA library obtained from *Xenopus laevis* oocytes and probes derived from the known sequences of the human and *Drosophila* genes, a cDNA coding for the $\alpha$-subunit of the *X. laevis* casein kinase II was isolated. The coding sequence of this clone determines a polypeptide of 350 amino acids. The *X. laevis* sequence is 98% identical to the human and rat proteins in the first 323 amino acids. Using the polymerase chain reaction to generate a 370-nucleotide-long probe, it was possible to clone and sequence a cDNA of 900 nucleotides that coded for the *X. laevis* $\beta$-subunit of casein kinase II. The derived protein sequence is 215 amino acids long and again shows an extraordinary degree of conservation with other species.

1. INTRODUCTION

Casein kinase II (CKII) is a protein kinase that is widely distributed in eukaryotes and that is not regulated by cyclic nucleotides, Ca$^{2+}$ or phospholipids (for review see [1]). The interest in this enzyme has increased recently due to reports that demonstrate that CKII phosphorylates several oncogenes and antioncogenes [2--4], transcription factors [5,6], enzymes that participate in nucleic acid synthesis [7,8] as well as other important proteins [9-11]. In addition, the work of several laboratories has demonstrated that the activity of CKII fluctuates during the cell cycle and seems to be regulated by mitogenic signals [12--14].

The CKII enzymes are tetramers of the $\alpha_2\beta_2$ or of $\alpha\alpha'\beta\beta'$ configuration. The cDNAs for the $\alpha$, $\alpha'$- and $\beta$-subunits of several mammalian species and of *Drosophila*, yeast and *C. elegans*, have been cloned and sequenced [15--19]. These sequences show a high degree of conservation.

Previous work from our laboratory has demonstrated that CKII is the most abundant protein phosphorylating activity present in the nuclei of *Xenopus laevis* oocytes and we have described its inhibition by nucleic acids and acidic peptides [20--22].

In this report, we present the sequence of the cDNAs coding for the $\alpha$- and $\beta$-subunits of the CKII of *X. laevis*. These results demonstrate a remarkable degree of conservation of the sequence of these protein subunits in such widely separated species as humans and amphibians.

2. MATERIALS AND METHODS

Oligonucleotides were synthesized with an Applied Biosystems 380A DNA Synthesizer in our laboratory. DNA sequences were determined using the method of Sanger et al. [23] and vectors M13mpl8 and M13mpl0 as described in the 'Sequenase' manual provided by U.S. Biochemical Corporation.

2.1. PCR amplification

PCR amplification was carried out as described [24] using Taq polymerase purchased from Promega. A *X. laevis* oocyte cDNA library constructed in the vector Agt10 was kindly donated by Dr. D.A. Melton of Harvard University [25].

2.2. Screening for the $\alpha$-subunit

The Agt10 library was screened for the cDNA coding for the $\alpha$-subunit of *X. laevis* CKII with several probes. Initially an oligonucleotide probe was synthesized based on the *Drosophila* alpha subunit gene [15]. This oligonucleotide had the sequence:

5′-GATAATGTGAGCTGGGATGTATGTCGCTGGCCAGCATGATC-3′

and was labelled with polynucleotide kinase. As described in the text, the clones isolated with this probe were truncated at the 5′ end. The second screening that yielded clones with the full coding sequence of the *X. laevis* $\alpha$-subunit employed two probes in replicate plating. The first probe was a 128 base-pair fragment corresponding to the 5′ end of the coding region of the human $\alpha$-subunit of CKII [26]. This fragment was generated by PCR amplification of human cDNA library with the primers:

upstream: 5′-ATGTCGGGGACCCGCG-3′

downstream: 5′-CGAACCAGCTGGTATGCAG-3′
The second probe was a fragment derived from the truncated gene and contained 439 nucleotides which corresponded to the sequence between nucleotide + 104 and nucleotide + 543 of the sequence given in Fig. 1.

Both probes were labelled by random priming. The screening methods were essentially as described by Maniatis et al. [27].

2.3. Screening for the β-subunit

The Agt10 X. laevis oocyte cDNA library was screened with a probe generated by PCR amplification of the same library using two primers derived from the nucleotide sequence of the gene coding for the human β-subunit of CKII [24]. The two primers used were:

5'-CGTGGCAATGAGTTCTTCGTG-3'
5'-ACATCCATGCACTTGGGCCA-3'

The probe generated in this fashion was 378 nucleotides long and corresponded to the sequence between nucleotide + 104 and nucleotide + 543 of the sequence given in Fig. 1. This probe was also labelled by random priming. The positive clones obtained were purified through secondary and tertiary screening as described in Maniatis et al. [27].

2.4. Reagents

General chemicals and reagents were of analytical grade, obtained from commercial suppliers. [γ-32P]ATP (7000 Ci/mmol) was purchased from ICN and [32P]dATP-α-S (>1000 Ci/mmol) from Amerham. Restriction enzymes, M13 cloning kit and special buffers, were purchased from Promega Biotech (Madison, WI). Nitrocellulose was purchased from Schleicher and Schuell.

3. RESULTS

3.1. Cloning of the cDNA coding for the α-subunit

Using an oligonucleotide probe synthesized on the basis of the sequence determined for the α-subunit of the D. melanogaster gene [15], a Agt10 cDNA library of X. laevis oocytes was screened. Among the positive clones detected, two were purified through quaternary screening. These clones were inserted into a M13mp18 vector and sequenced by the method of Sanger et al. [23]. Both of these clones were found to code for the α-subunit of X. laevis CKII but they differed in length. One contained a 3' non-coding segment of more than 1000 nucleotides while the other only extended 65 nucleotides after the stop codon. The longer fragment contained the polyadenylation signal and a poly-A segment. However, both cDNA clones were truncated at

Fig. 1. The nucleotide and predicted amino acid sequence of the α-subunit of X. laevis casein kinase II. The Met initiation codon is at nucleotide 55 and the termination codon TGA is at nucleotide 1105.
an identical position which corresponded to nucleotide + 108 of the human αCKII subunit. This finding made it necessary to return to the cDNA library and re-screen, using two probes in replicate plating. The first probe corresponded to a fragment of the previously cloned truncated gene containing 439 base pairs, and the second probe was a 128 base pair PCR product from a human cDNA library and corresponded to the missing coding segment of the truncated genes. Only 2 out of the 5 clones analyzed were positive with both probes indicating that there is a relatively high incidence of truncated cDNAs for this gene in this cDNA library. However, it is interesting that other cDNA libraries seem to have a high number of truncated clones of this gene [28].

One of the doubly positive clones was purified, inserted into a M13mp10 vector and sequenced as described previously. Fig. 1 gives the nucleotide sequence of the coding region of this clone with short non-coding segments at the 5' and 3' ends. The sequence found in this clone was identical to the corresponding sequences previously determined in the truncated cDNAs. This figure also shows the derived amino acid sequence coded by this clone. The α-subunit of *X. laevis* CKII is a protein of 350 amino acids and a calculated Mr of 41,479 kDa.

A remarkable degree of sequence conservation is observed by comparing the amino acid sequence of the α-subunit of *X. laevis* CKII with the sequences of the α-subunits of other species (Fig. 2). In the first 323 amino acids, the *X. laevis* α-subunit sequence is identical to the human and rat sequences except for 4 conservative changes.
Fig. 3. The nucleotide and predicted amino acid sequence of the β-subunit of *X. laevis* casein kinase II. The Met initiation codon is at nucleotide 55 and the termination codon TGA is at nucleotide 700. The underlined bases correspond to the regions in the human CKII gene used to design the primers for PCR.

Fig. 4. Comparison of the amino acid sequence of the β-subunit of *X. laevis* casein kinase II with those of human, bovine, drosophila and chicken. The dots indicate probable p34^δ^-2 phosphorylation sites at amino acids 145 and 209.
shown in Fig. 3. The β-subunit of X. laevis CKII is a polypeptide of 215 amino acids with a calculated Mr of 24,960 Da.

Again, the gene for this subunit shows an amazing degree of sequence conservation. At the nucleotide level comparison of the coding regions of human and X. laevis shows 125 bases changed out of a total of 645. Almost all of these changes are in the third place positions or mutations that maintain codons for the same amino acid. Fig. 4 compares the amino acid sequences of X. laevis with those of 4 other species. It can be seen that the X. laevis β-subunit is identical to the human polypeptide in the first 193 amino acids and in the remaining of the molecule it only has 2 different amino acids. The X. laevis and chicken subunits only differ in 1 amino acid. This extraordinary conservation seems more remarkable because of the fact that this subunit is not known to carry out any catalytic function, its only known activity is the capacity to stimulate the phosphorylation in the human β-subunit would be [32,33]. According to Litchfield et al. [33], the site of phosphorylation in the human β-subunit may be phosphorylated by the p34cdc2 kinase would be in [34].

Recently, there have been two reports that indicate that the β-subunit may be phosphorylated by the p34cdc2 kinase that regulates key transitions in the cell cycle [32,33]. According to Litchfield et al. [33], the site of phosphorylation in the human β-subunit would be serine-209. However, Mulner-Lorillon et al. [32], who used purified X. laevis CKII in vitro found that p34cdc2 phosphorylated the β-subunit in an unidentified threonine residue. Since serine-209 is present in the same position in the X. laevis subunit as in the human, these observations would suggest that the phosphorylation of the X. laevis by the p34cdc2 kinase would be in a different position. This phosphorylation site may correspond to threonine-146 which is in a sequence that fulfills the consensus required by the p34cdc2 kinase [34].

Acknowledgements: We gratefully acknowledge the donation of the Agt10 cDNA library by Dr. D. Melton. We also acknowledge the continued advice and helpful discussions with Dr. Juan Olate. This work was supported by the Council of Tobacco Research, the International Center for Genetic Engineering and Biotechnology and by FONDECYT-Chile.

REFERENCES