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The genomic structure of two protein kinase CK2α genes of *Xenopus laevis* and features of the putative promoter region

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Abstract

Protein kinase CK2 is an enzyme that is ubiquitous in eukaryotes. This enzyme, composed of catalytic (α and α′) and regulatory (β) subunits, is responsible for the phosphorylation of a large number of proteins and is implicated in cell division. Genomic clones coding for the CK2α subunit of *Xenopus laevis* have been isolated. Initial restriction enzyme profiles and subsequent PCR analysis and DNA sequencing indicated that these genomic clones correspond to two different genes. The two genes are highly homologous in the regions of the coding sequence (only 3 amino acid differences) but differ considerable in their intron sequences and lengths. Gene 1 corresponds to the cDNA of *Xl*CK2α which had been previously isolated and described. The genomic clone for this gene was truncated. Gene 2 contains the entire coding region for CK2α subunit as well as a fragment of 6.4 kb of the 5´upstream region. The exon/intron boundaries of both genes obey the GT/AG rule with the exception of intron V where the less common GC/AG is seen. Comparison of the size of ten coding exons and sites where these are interrupted by introns shows strong conservation with respect to the human CK2α gene. RT-PCR analysis of mRNAs from *X. laevis* ovary, oocytes and early embryos using a specific primer for gene 2 demonstrated that this gene is expressed in these tissues and cells. Analysis of transcription start sites using 5′RACE and RNA from stage VI oocytes demonstrated that there are multiple start sites in the *Xl*CK2α mRNA. It was also seen that a noncoding exon 1 is present 4 kb upstream of the translation start site and that alternate splicing occurs in gene 2 to give exon 1 of different lengths. Sequencing of the entire upstream genomic region of gene 2 revealed that there are regions of homology to the sequence of exon 1 of the human CK2α gene. Other sequences with consensus to transcription factor binding sites that are seen in the promoter region of human CK2α are also found in the *X. laevis* CK2α gene 2. These sites include Ets1, E2F, CCAAT and GC rich regions. No canonical TATA motif is observed. (Mol Cell Biochem **227**: 175–183, 2001)

Key words: casein kinase 2, transcriptional regulation, CDK2 promoter, Ets1 site

Introduction

Protein kinase CK2 (also known as casein kinase 2) is ubiquitous in eukaryotes and is responsible for the phosphorylation of more than 200 different protein substrates [1–3]. There is a large body of evidence that indicates that CK2 is involved in cell division. The finding that CK2 content, both at the protein and mRNA levels, is considerable higher in dividing and tumor tissues is coincident with this functional involvement and raises the issue of how the transcription of the genes coding for the catalytic (α and α′) and the regulatory (β) subunits of this enzyme is regulated. On the other hand, it is clear that the regulation of the expression of these genes is important, as seen by the fact that overexpression of CK2α in lymphoid cells of transgenic mice [4] or cattle infected with *Theileria parva* [5] can lead to lymphomas in these animals.

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Pyerin's laboratory has contributed considerable to our knowledge of the genomic structure of the genes coding for CK2α and CK2β in humans and also regarding some of the features present in their promoter regions that may contribute to the transcriptional regulation of these genes [6–8]. However, there has been a dearth of information about the structure of the CK2 genes in species other than human and about the conservation or divergence of their promoter regions throughout evolution.

In this report, we present studies that describe the presence of two different genes that code for CK2α in *X. laevis* and that are expressed in ovary and early embryos. Analysis of genomic clones of these two genes has allowed us to determine their structural features. It is shown that while they are nearly identical in the amino acids of the protein coded by their exons, they differ significantly in the sequence and length of their introns. A study of the transcriptional start sites using mRNA isolated form early *X. laevis* embryos demonstrate that there are at least two start sites for gene 2 and that both genes contain a noncoding first exon and this exon 1 contains regions of great homology to the corresponding first exon in human CK2α. Another important finding described here is the presence of an Ets1 site in the close vicinity of the start site of both CK2α genes of *X. laevis*. Ets1 sites have been found by Krehan *et al.* [8] and shown to be important for human CK2α transcription. The 5′ region of the XlCK2α gene also contains elements present in the *X. laevis* CDK2 gene promoter region and in the human Cyclin B gene promoter region. Several consensus sequences for the E2F transcription factor are also seen. Similar to the case of the human gene, *Xl*CK2α does not contain a canonical TATA box but does contain upstream CCAAT boxes and GC-rich regions.

Materials and methods

Cloning of genomic fragments of XlCK2α

A genomic library of *Xenopus laevis,* constructed in bacteriophage lambda EMBO4 vector, was a kind donation of Dr. Igor Dawid (NIH). Inserts were 15–20 kb cloned in a BamHI site. The library was screened by plaque hybridization with a nick-translated ^{32}P-labeled 1110 kb fragment corresponding to the entire coding region of *Xl*CK2α and which also included 40 nucleotides of the 5′ noncoding region. A total of 10^6 pfu were screened, 6 positive clones were isolated and secondary and tertiary screening was performed with the same probe and with two additional labeled fragments obtained by PCR and which represented nucleotides 105–293 and 568–728 of the *Xl*CK2α cDNA. In order to estimate the size and the presence of putative promoter regions in these clones, the clones were analyzed using restriction enzymes *EcoR*I, *Pst*I, *Hind*III and *Kpn*I (Promega) and the fragments were Southern hybridized with probes representing the entire coding region of *Xl*CK2α and a second 5′ terminal probe (nucleotides –40 to 169).

Partial sequencing of clones 1 and 2 was performed initially using primers of the λ vector sequence together with reverse primer **pC2**, (5′-CACGTGTGACTCATAGTCCC-AA-3′), designed from the 5′ coding region of *Xl*CK2α cDNA, (nucleotides 69–90 from the transcription start site). For complete sequencing of the upstream region of clone 2 (gene 2), restriction fragments generated by digestion with *Sal*I were subcloned in vector PUC18 (Pharmacia) and sequenced in both directions using vector primers and then internal primers designed from determined sequences. A fragment of 6.4 kb upstream of the ATG translation start site and part of exon 2 was sequenced (NCBI Genbank Accession number AY032954). Sequencing was performed using Applied Biosystems Model 377 automatic sequencer. All PCR reactions with genomic clones were performed with Expand Long Template PCR System (Roche Molecular Biochemicals).

PCR amplification of genomic clones 1 and 2 and comparison with products obtained with cDNA of XlCK2α

Genomic DNA from liver was prepared as described by Gustincich *et al.* [9]. A series of synthetic oligonucleotides designed from the sequence of *Xl*CK2α cDNA [10] were used as primers for PCR for the comparison of genomic DNA (liver) and the cDNA of CK2α (see Fig. 2), as follows:

p1, 5′-GTCTACAAAACGCTCTGCCT-3′;
p2, 5′-TGGCTTCAAAGACTTCACTG-3′;
p3, 5′-CCAGGATGATTATCAGTTAGT-3′;
p4, 5′-GCCAGGGTGATAATGTTGG-3′;
p5, 5′-CCAACATTATCACCCTGGC-3′;
p6, 5′-GTGGCAATAATCCAGTGCC-3′;
p7, 5′-TGGTAATCCACAAGCAGCTC-3′;
p8, 5′-GTCCGTGTTGCTTCCCG-3′;
p9, 5′-ACCAGCTGGTCGTAGTTATC-3′;
p10, 5′-CCACGCTTTAATGATATTCT-3′;
p11, 5′-CTTGTCCAGGAAATCCAGTG-3′;
p12, 5′-GACTGACCTGACATCATAC-3′.

Oligonucleotide primers were synthesized in the core facility of the University of Chile using an automated sequencer (ABI377).

Boundaries of exons/introns were determined by comparing the cDNA and genomic sequences. The remaining coding sequence of gene 2 was determined by PCR as well as direct sequencing.

Analysis of genomic sequence fragments for characteristic promoter sites was performed with TFMATRIX transcription

factor binding site profile database (E. Wingender, R. Knueppel, P. Dietze, H. Karas (GBF-Braunschweig). Searches for sequence similarities were performed using NCBI Blast 2 Sequences program.

Expression of XlCK2α

The primer used for detection of the specific expression of *Xl*CK2α gene 2 mRNA by RT-PCR using different tissues was **pαG2**: 5′-CTGCCCAGGATATCATTG-3′. Total RNA was prepared from *X. laevis* oocytes and embryos as given by Chomczynski and Sacchi [11] and was reverse transcribed using AMV reverse transcriptase (Promega) at 37° for 60 min using primer **pαG2** followed by PCR using primers **p8** and **pαG2**. In parallel and as control, reverse transcription was carried out with primer **p12**, followed by PCR with **p8** and **p12**. *X. laevis* oocytes and embryos were obtained as described [12].

Rapid amplification of 5′ mRNA ends (5′ RACE) was performed using a 5′,3′ RACE system (Boehringer Mannheim) and was used as suggested by the manufacturer. The reverse transcriptase step of the preparation used the CK2α gene-specific primer **pTrans** 5′-CAGAGGCAAAACTGGGCGGT-3′ and PCR performed with the supplied primer. RNA for these studies was prepared with Trisol (Gibco), using stage VI *Xenopus laevis* oocytes. The cDNA products were cloned in PUC19 and sequenced.

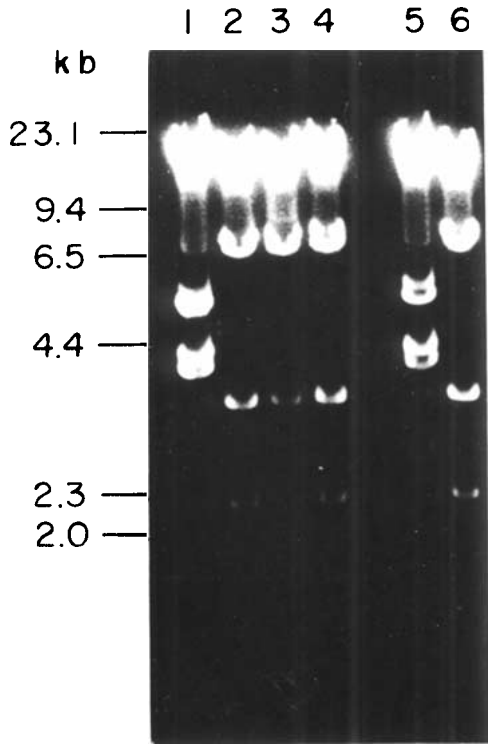

Fig. 1. Comparison of the EcoRI restriction patterns obtained using selected genomic clones which hybridize with *Xl*CK2α cDNA. DNA from 6 genomic clones positive for CK2α was purified and digested with EcoRI and analyzed by 0.7% agarose gel electrophoresis.

Results and discussion

Isolation of genomic clones coding for X. laevis *CK2α: Evidence for the presence of two different genes*

A genomic gene library constructed in λEMBL4 was screened as given in Materials and methods using as probe a 1100 base pair fragment which included the coding region and 40 nucleotides of the 5′ upstream region of the *X. l.* CK2α cDNA previously isolated. [10]. A total of six positive clones were obtained after tertiary screening in which specific probes representing the 5′ and 3′ regions were included in the analysis. Southern analysis with two distinct probes from the amino and carboxyl coding regions of CK2α showed that two of these clones (clones 1 and 5) did not interact with the probe representing the 3′ end of the gene (not shown). Digestion of all six clones with EcoRI demonstrated that clones 1 and 5 gave similar patterns but a different digestion pattern was seen with the other 4 clones (2, 3,4 and 6) which were all clearly related (Fig. 1).

The possibility that these two sets of genomic clones might represent two different CK2α genes was explored using PCR

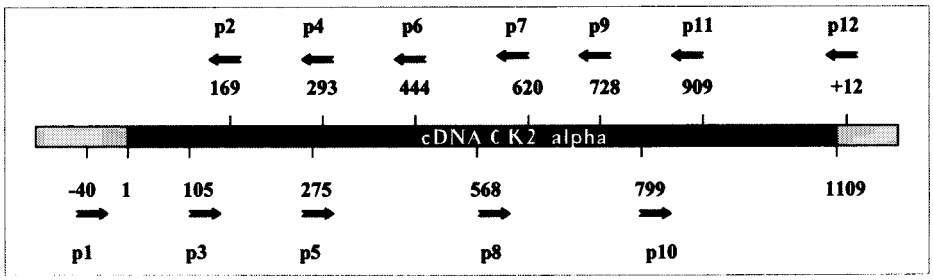

Fig. 2. Schematic representation of the nucleotide primers used for the analysis of the genomic structure of *Xl*CK2α and for sequencing of the coding regions. Primer sequences are given in Materials and methods.

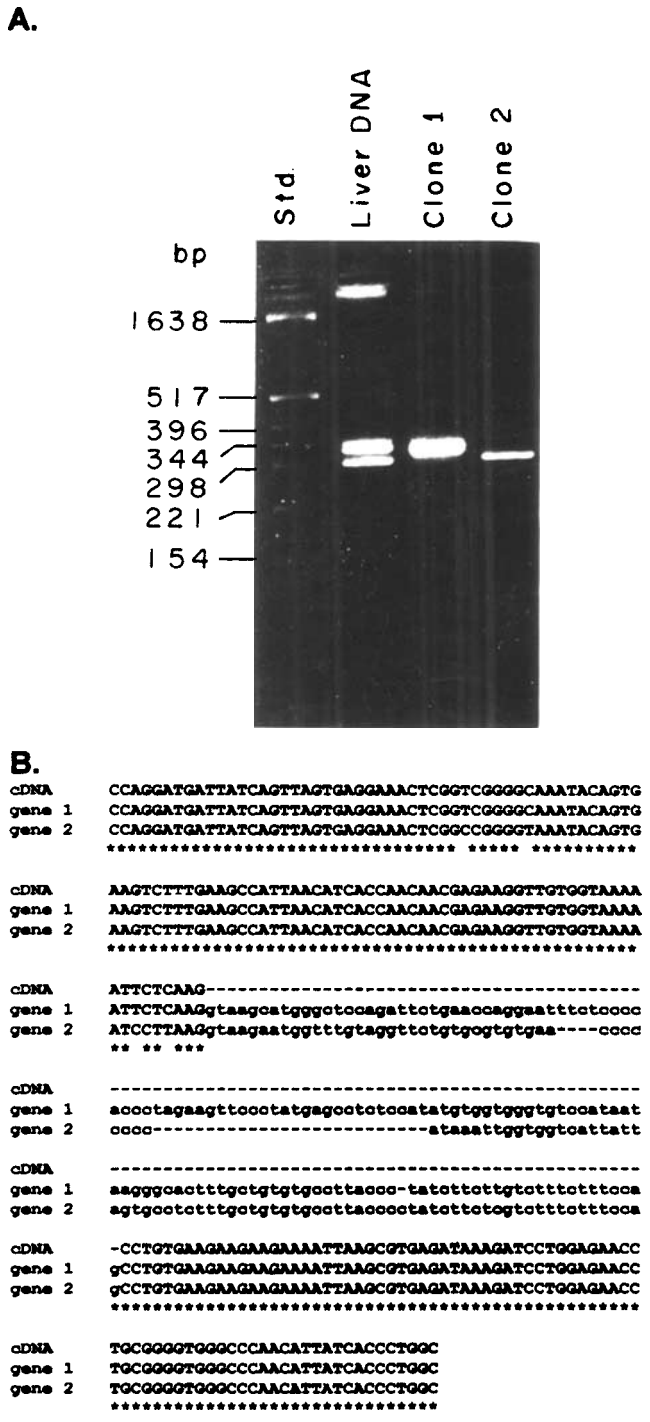

Fig. 3. PCR products and nucleotide sequences obtained with primers p3 and p4 using *X. laevis* genomic DNA or genomic clones 1 and 2 for *Xl*CK2α. (A) Primers p3 and p4 were used with 300 ng liver DNA or 20 ng genomic clone 1 and 2 as templates for PCR amplifications and the products analyzed by agarose gel electrophoresis. The first lane shows DNA standards. (B) Nucleotide sequence of the two *X. laevis* CK2α genomic clone fragments are compared with the cDNA of *X. l.*CK2α. PCR amplification and sequence determination was performed with primers p3 and p4. Asterisks indicate identity between the three fragments. Capital and lowercase letters are exon and intron sequences, respectively.

with sets of primers designed from the coding regions of the CK2 cDNA. Figure 2 shows a scheme of the primers used. These PCR analyses were also carried out with genomic DNA isolated from *X. laevis* liver. Figure 3A shows that using primers 3 and 4, the PCR products obtained with rat liver DNA is a mixture that is resolved into two different products when either clone 1 or clone 2 DNA is used as template. Sequencing of the two PCR products obtained from clones 1 and 2 demonstrated that clone 1 is identical to the cDNA previously obtained and that the sequences of clones 1 and 2 are very similar within the coding regions but differed significantly in the base composition and length of the introns (Fig. 3B). PCR analyses with set of primers covering the entire length of the cDNA indicated that clone 2 contains the entire coding sequence for CK2α and that the coding exons and the 10 intervening introns correspond to 8 kb in length. The sequence observed for clone 1 corresponds to about one half of the CK2α coding region. These results strongly support the presence of two clones corresponding to two separate genes coding for *X. laevis* CK2α (henceforth referred to as genes 1 and 2, derived from clones 1 and 2, respectively). The coding region of the two genes as present in these two clones is shown in Fig. 4. Comparison of the sequence of these two forms to that of the cDNA previously isolated [10] demonstrated that this cDNA was clearly equivalent to the truncated gene 1. The deduced amino acid sequences coded for by gene 1 and gene 2 are shown in Fig. 5, where a total of 3 conserved amino acid changes are found within the studied segments.

It is interesting to point out that, contrary to what has been observed with most eukaryotes studied, no CK2α′ species has been found in *Xenopus laevis*. It seems possible, however, that the second CK2α gene which lacks the distinctive features of the CK2α′ and is clearly a CK2α form, may be fulfilling the function of the CK2α′ gene which has been shown to be specifically involved in spermiogenesis through experiments in knock-out mice [13]. However, the tetraploidy of *X. laevis* frequently results in the existence of 2 genes coding for a single protein [14].

Exon-intron structure of the coding region of Xl*CK2α genes*

Partial sequencing of PCR products using *X. laevis* liver DNA as template and primers representing the entire *Xl*CK2α cDNA enabled us to define the overall exon-intron structure of the coding region of the *Xl*CK2α genes. Table 1 summarizes the data for gene 2 and it may be seen that the intron/exon boundaries conform to the GT/AG rule, with the single exception of intron V where GC/AG is found. As seen in Figs 4 and 5 above, there is nearly complete identity between the coding regions of genes 1 and 2. These genes, which are very different in intron size and sequence, possess absolute

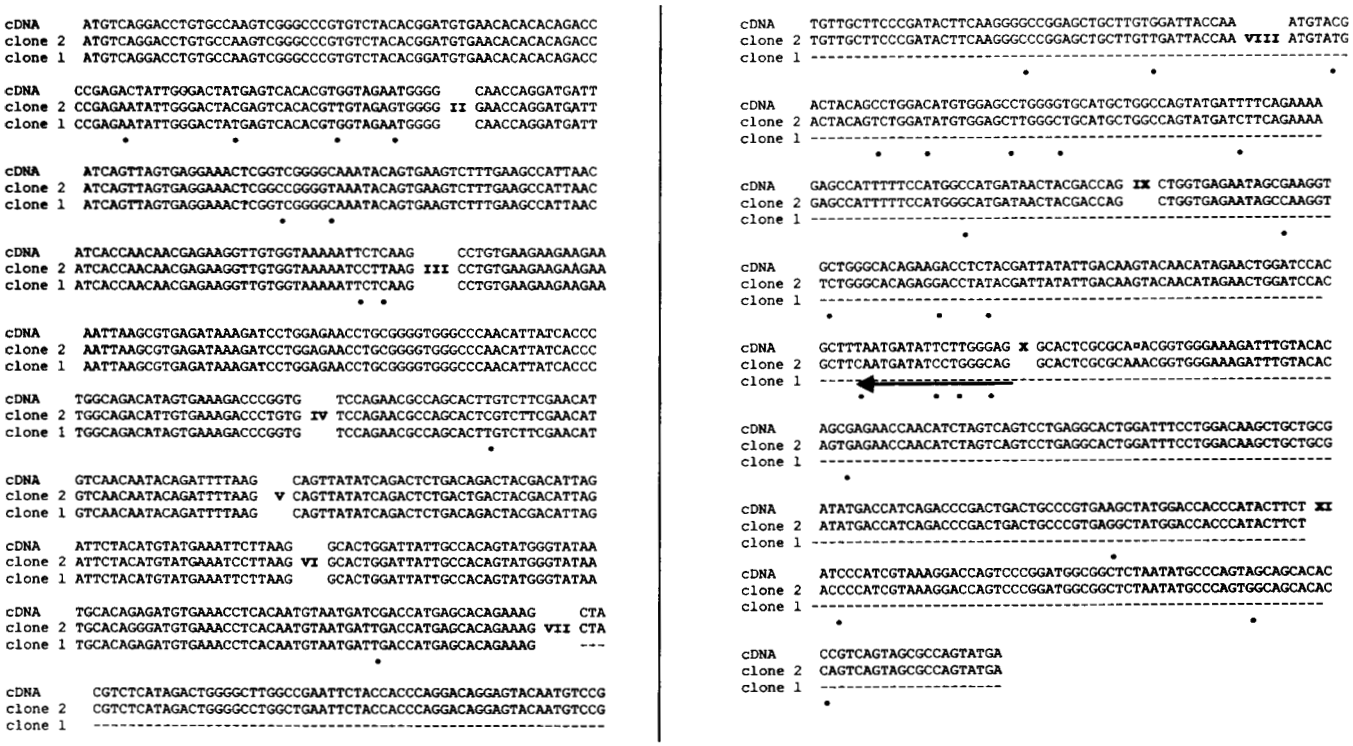

Fig. 4. Comparison of the coding sequences of the genomic clones 1 and 2 with the *Xl*CKα cDNA. Roman numeral inserts indicate the positions of intron segments; dots below the sequence indicate variations in the clone 2 sequence with respect to the *Xl*CK2α cDNA; dashed lines indicate the region deleted in clone 1. The arrow indicates the position of primer pαG2 designed to detect exclusively gene 2 and used to analyze the expression of this gene.

Table I.
Exon-intron structure and splice sites of CK2α of X. laevis.

Exon No.	Exon size (bp)	SEQUENCES AT JUNCTIONS 5' donor 3' acceptor	Intron No.	Intron size (bp)
1.	85	CAG/gtgtgg--//--cag/CGA	I	4203
	115	CTT/gtacgt--//--cag/CGA		4173
2.	189	GGG/gtaagt--//--cag/CAA	II	(4000)
3.	112	AAG/gtaaga--//--cag/CCT	III	112
4.	102	GTG/gtaagt--//--cag/TCC	IV	81
5.	51	AAG/gcgagt--//--cag/CAG	V	(1700)
6.	60	AAG/gtaatt--//--cag/GCA	VI	100
7.	84	AAG/gtaagt--//--cag/CTA	VII	80
8.	111	CAA/gtatgt--//--cag/ATG	VIII	155
9.	102	CAG/gtgaga--//--cag/CTG	IX	(1700)
10.	101	CAG/gtaagt--//--cag/GCA	X	96
11.	149	TCT/gtaagt--//--cag/ACC	XI	249

Capital letters denote exon sequences and lowercase letters intron sequences. Intron sizes were determined by sequence analyses except (in parenthesis) when estimated by the size of the PCR product.

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x.l.αcDNA    MSGPVP-SRARVYTDVNTHRPRDYWDYESHVVEWGNQDDYQLVRKLGRGKY 50
x.l.Gene2    .......................E.............................
H.s.CK2α     .......................E.............................
H.s.CK2α'    .P..AAG.......AE..SL.S.E.....A..PS..................

x.l.αcDNA    SEVFEAINITNNEKVVVKILKPVKKKKIKREIKILENLRGGPNIITLADI 100
x.l.Gene2    ................................................
H.s.CK2α     ................................................
H.s.CK2α'    .........NS..R.................V.........T...K.I.T

x.l.αcDNA    VKDPVSRTPALVFEHVNNTDFKQLYQTLTDYDIRFYMYEILKALDYCHSM 150
x.l.Gene 2   ................................................
H.s.CK2α     ................................................
H.s.CK2α'    ......K.......YI..........I...F........L.........

x.l.αcDNA    GIMHRDVKPHNVMIDHEHRKLRLIDWGLAEFYHPGQEYNVRVASRYFKGP 200
x.l.Gene 2   ................................................
H.s.CK2α     ................................................
H.s.CK2α'    ................QQK..............A..........SY...

x.l.αcDNA    ELLVDYQMYDYSLDMWSLGCMLASMIFRKEPFFHGHDNYDQLVRIAKVLG 250
x.l.Gene 2   ................................................
H.s.CK2α     ................................................
H.s.CK2α'    ............................R......Q..............

x.l.αcDNA    TEDLYDYIDKYNIELDPRFNDILGRHSRKRWERFVHSENQHLVSPEALDF 300
x.l.Gene 2   ........E.....................................
H.s.CK2α     ................................................
H.s.CK2α'    ..E..G.LK..H.D...H......Q.......N.I....R.........L

x.l.αcDNA    LDKLLRYDHQTRLTAREAMDHPYFYPIVKDQSRMGGSNMPSGSSTPVSSA 350
x.l.Gene 2   ................................................
H.s.CK2α     ..................................S.S..G..-......
H.s.CK2α'    ..........Q....K...E......V..E..------Q.CADNAVL..G

x.l.αcDNA    SMMSGISTVPTPSALGSLAGSPVISATNTLGTPVAAAAGATQ-392
x.l.Gene 2   ...........Q.................
H.s.CK2α     N......S.....-..P.......A.A.P..M..P.....Q.
H.s.CK2α'    LTAAR
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Fig. 5. Comparison of the predicted amino acid sequences of *Xl*CK2α cDNA with *Xl*CK2α gene 2 and with CK2α and α′ of *H. sapiens*. Dots indicate identity with *X. laevis* and dashes indicate that the amino acid is absent from the corresponding sequence.

homology in the coding exon sizes and in all of the donor and acceptor splice signals, as far as has been determined (gene 1 clone being truncated at exon 8 and gene 2 at exon 11). A comparison of the exon size of the coding structure of *Xl*CK2α with that found in the human CK2α gene [7] shows that the positions of RNA processing are also conserved between these two species, giving rise to exons of the same size and with similar boundaries.

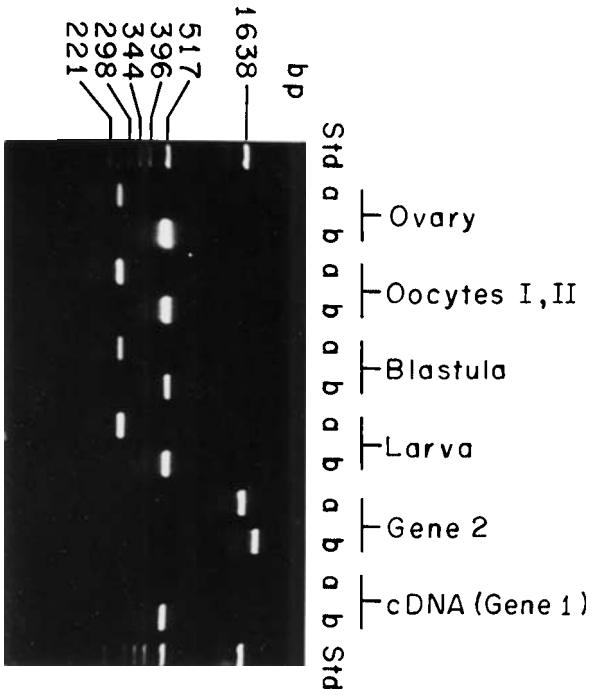

Fig. 6. Expression of *Xl*CK2α gene 2 in *X. laevis*. Total *X. laevis* RNA from oocytes, ovary and embryos of stages 8 (blastula) and 34 (larva) was used for reverse transcription using primer pαG2 (specific for gene 2, see Fig. 4) or with primer 12 (common for cDNA of both gene 1 and 2) followed by PCR using: lanes a – primers p8 and pαG2; lanes b – primers p8 and p12. Controls were performed using cDNA of gene 1 and DNA from gene 2.

Expression of the two X l*CK2α genes*

The human genome contains a silent pseudogene for CK2α [15]. It was pertinent therefore to determine whether the two *Xl*CK2α genes observed were expressed. Since gene 1 is identical to the cDNA isolated from oocytes, it was clear that this gene is actively expressed in this type of cell. To detect the presence of the specific mRNA corresponding to gene 2, a primer was designed for a region which contained a cluster of structural differences between gene 1 and gene 2. The horizontal arrow in Fig. 4 indicates the region used for the primer sequence. Using this primer together with a reverse primer (primer 8 of Fig. 2), gene 2 gave a PCR product of the expected length (1700 bp) while gene 1 did not act as a template for amplification. RT-PCR of total mRNA isolated from ovary, oocytes an early embryos (blastula and larval stages) demonstrated that RNA transcribed from gene 2 was present in all of these cells and tissues (Fig. 6). Sequence determinations of the RT-PCR products confirmed that the RNA corresponded to gene 2 of *Xenopus laevis* CK2α (not shown).

The 5′ upstream region of the X. laevis *gene 2*

A 6.4 kb fragment corresponding to the region upstream of the 5′ coding end of gene 2 was sequenced as described in Materials and Methods. Using mRNA from X. laevis stage VI oocytes and RT-PCR (5′RACE), experiments were carried out to determine the transcription start sites of the CK2α genes and to relate this information to the genomic structure. Figure 7A. shows the results of these studies and indicates the transcription start sites obtained by the RT-PCR analysis. It is seen that there are two principle start sites (designated as nucleotides 1 and 28) and that a nontranslated exon is present within this fragment. In addition, examination of the RT-PCR products also indicated that two distinct 5′-donor sites exist within exon 1, a fact that indicates that alternative splicing of the transcripts would produce exon 1 of different lengths (see Table 1). Examination of the sequences present in a region 2000 bases upstream of the transcription start sites of gene 2 indicates that this gene is similar in structure to the so-called housekeeping genes in that it lacks the typical TATA box and CCAAT signals proximal to a unique start site. Searches for canonical sequences in this segment reveal several elements that match those of transcription factor binding sites, including, besides those indicated in the figure 7A, sites for factors YY1 (–85), SRY (–103, –97: Sex Determination Region Y gene) and upstream CCAAT boxes. The interpretation of the importance of these elements awaits further study.

Identity of the sequence of one of the 5′ RACE cloned products with that of a sequence present in the nontranslated segment near the initiator ATG codon present in the cDNA of gene 1 indicate that this RT-PCR product corresponded to gene 1 mRNA. This RT-PCR product also contains regions with strong homology to those found in exon 1 of gene 2 (Fig. 7B).

In Fig. 7B a comparison is made of the noncoding exon 1 of human CK2α [7] with its counterparts from *X l*CK2α genes 1 and 2. Significant stretches of homology exist in this CK2α exon sequence of these two species (double-underlined in the human sequence). It should be noted that the homology includes the Ets-1 site which has been identified by Pyerin's laboratory [8] as a major determinant of the human CK2α promoter strength. *X. laevis* gene 1 transcription starts precisely in the Ets1 site while *Xl*CK2α gene 2 contains that signal at –20 nucleotides of the first detected start site. It is very interesting that the human CK2β gene also has an Ets1 site very close to the transcription start site [6]. The proximity of Sp1 sites to the Ets1 site has been judged also to have relevance in the promoter function [8]. In the *Xl*CK2α genes there are several GC-rich segments localized near the transcription start site and these regions are both within and near the regions of homology of the Sp1 sites seen in the human gene. These findings suggest a possible coordination

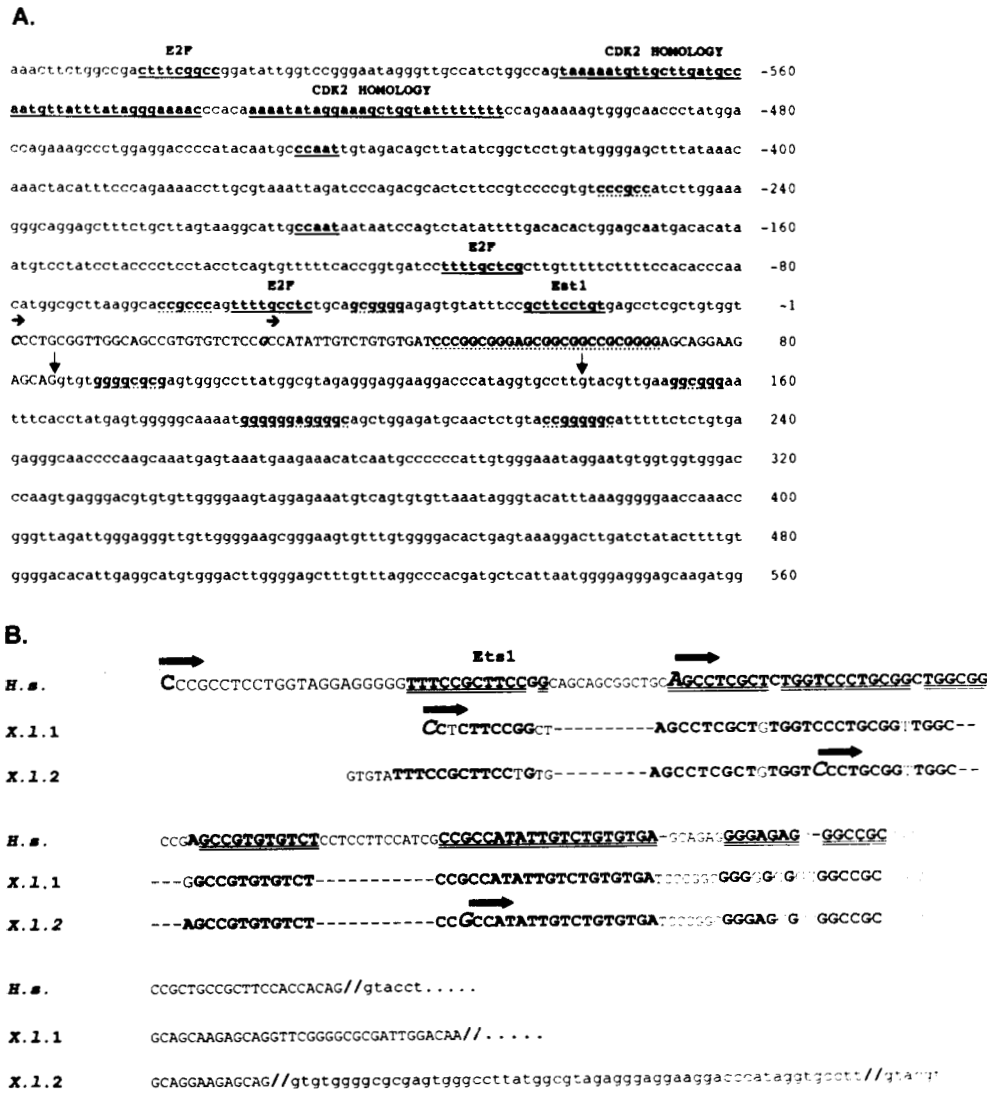

Fig. 7. (A) DNA sequence of the 5′-flanking region of the *X. laevis* CK2α gene 2. Horizontal and vertical arrows show two observed transcription start sites and two alternative splice sites, respectively. Capital letters indicate exon 1 (up to the first splice site) and lowercase letters show intron I. A region of homology to a sequence present in the CDK2 promoter region as well as putative transcription factor binding sites are indicated and underlined. GC-rich regions are underlined by dots. (B) Homology of the noncoding exon 1 of the CK2α genes from human and *X. laevis*. The complete sequence of exon 1 of *H. sapiens* is given for comparison with the two CK2α genes from *X. laevis*. Horizontal arrows show transcription start sites. Identical structures are indicated by bold capital letters with double underline of the *H. sapiens* sequences. Gaps are shown by dashes, lowercase letters show introns and slashes represent splice sites. The presence of an Ets1 site in *H. sapiens* and a small segment of *X. laevis* gene 2, upstream of exon 1, are shown for comparison.

in the regulation of the transcription of the two subunits of CK2 and conservation of the regulatory signals that control the expression of CK2 throughout evolution. It may also be relevant that within the region that is homologous to the human CK2α promoter, there is a 90 to 95% homology in a 22 base segment (nucleotides +4 to +25 and –1 to –23 of the *Xl*CK2α gene 1 and gene 2, respectively) to a sequence in the promoter region of human cyclin B1 gene. It is also noteworthy that the putative promoter region of XlCK2α gene 2 contains a region of high homology to a region that is present in the promoter region of *X. laevis* CDK2 (region –505 to –541 Fig. 7A.). This observation suggests that these two kinase which are closely related structurally in the protein kinase family [16] and which are both involved in cell division, might also share similarities in the regulation of the transcription of their genes.

Acknowledgements

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