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Expression of the subunits of protein kinase CK2 during oogenesis in *Xenopus laevis*

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Northern-blot analysis of RNAs from different tissues demonstrated that the mRNA for the protein kinase CK2α subunit is very abundant in the ovary of *Xenopus laevis*. The competitive reverse-PCR technique has been used to quantitate the mRNA for both CK2α and CK2β subunits during oogenesis. The results obtained using eight different animals consistently show an increment of 2–3-fold in the mRNA for both subunits in vitellogenic oocytes (stages II–VI). Each stage-VI oocyte contains approximately 5×10^{-7} molecules CK2α mRNA and 1×10^{-7} molecules CK2β mRNA. These amounts are considerably higher than many other mRNAs analyzed in these cells. Activity measurements of CK2 using casein or a specific model peptide revealed increments of about 10–12-fold during oogenesis, and also indicated that the amount of enzyme in the nucleus accounted for 15–30% of the total enzyme in the oocyte at all stages. Western-blot analysis of CK2α indicated that the amount of this protein subunit also increased during oogenesis in a parallel fashion with the increment of enzymic activity.

Keywords: casein kinase 2; mRNA quantification; competitive reverse PCR; protein phosphorylation.

Protein kinase CK2 (also known as casein kinase 2 or CKII) is a ubiquitous Ser/Thr protein kinase that is widely distributed in eukaryotic cells, both in cytoplasm and in the nucleus [1–2]. This enzyme has been shown to phosphorylate more than 100 different protein substrates, including DNA and RNA polymerases, DNA topoisomerase II, DNA ligase, jun, fos, myc, max, myb, p53, Rb, calmodulin, $p34^{cdc2}$ and the regulatory subunit of protein kinase A.

CK2 is a heterotetramer with the composition $\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$. The α and α' subunits contain the catalytic activity while the β subunits are regulatory in the sense that they increase the activity of CK2α with most protein substrates and also affect the specificity towards these substrates.

Convincing evidence links the quantitative expression of this enzyme to cell proliferation, since it has been found that both the mRNA and the protein subunits of the enzyme are more abundant in the rapidly dividing cells of embryonic tissues, in transformed cells in culture and in isolated tumors [3–5]. Preferential expression of the mRNAs coding for both subunits in developing nervous tissue has been observed in embryos of mice, zebrafish and *Xenopus laevis* [6, 7] (Wilhelm, V., Mayor, R., Allende, C. C. and Allende, J. E., unpublished results). Recent data has provided evidence that CK2α can be a proto-oncogene in mouse lymphocytes [8].

Oogenesis is a key process in cell differentiation which can be conveniently studied using the ovaries of mature *X. laevis* females since this tissue contains oocytes at the six different stages of oogenesis [9]. Classical as well as more recent studies [10–12] have established that oocytes in early or previtellogenic stages of oogenesis (stages I and II) transcribe the majority of the mRNAs that are found in the full-grown cell (stage VI) There are, however, exceptions to this generalization where certain specific mRNAs continue to increase throughout oogenesis [13] or decrease several fold [14]. During stages III–IV, in contrast, these cells principally transcribe ribosomal RNA [15].

The important role that CK2 seems to play in both embryonic and proliferating cells prompted us to study the expression of the genes coding for the α and β subunits of protein kinase CK2 during oogenesis. The cDNAs coding for both of these subunits present in *X. laevis* oocytes have been cloned, expressed and the recombinant proteins characterized [16, 17].

In this communication, we present results which demonstrate that the mRNAs coding for the α and β subunits of CK2 increase significantly at stages II–IV of oogenesis. It is further shown that the protein encoded by the CK2α subunit gene as well as the enzymic activity of CK2 also increases significantly during the more advanced stages of oogenesis.

MATERIALS AND METHODS

Materials. Oligonucleotides and synthetic peptides were synthesized by Oligopeptido Chile, using instruments from Applied Biosystems. Radionucleotides were from Amersham or ICN. Unlabeled nucleotides were from Boehringer. Promega supplied the Western-blot alkaline phosphatase system, restriction enzymes, DNase Q, Ta, reverse transcriptase and RNAsin.

Protein kinase A inhibitor protein, protease inhibitors, β-dephosphocasein, heparin and general reagents were from Sigma. Nuctrap columns were from Stratagene. Nytron membranes were from Schleicher & Schüll and nitrocellulose membranes from Sigma. Prestained molecular-mass standards were from BioRad.

Methods. *Oocytes.* Ovarian fragments were removed surgically from adult *X. laevis* females using hypothermic anesthesia and kept in Barth's medium [18]. For separation of different

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Abbreviations. CK2, protein kinase CK2 or casein kinase 2; CK2α, the α subunit of CK2; CK2β, the β subunit of CK2.

Enzyme. CK2 (EC 2.7.1.37).

stage oocytes, these fragments were agitated gently in 0.2% collagenase in Barth's solution for 1 hour, then manually separated into the six stages of oogenesis [9]. In some experiments, oocytes of stages I and II were combined. Oocytes were transferred to a solution of 5 mM Tris/HCl, pH 7.5, containing 10 mM $MgCl_2$ and, after 15 min, the remaining follicle cells were removed manually, and oocytes immediately homogenized.

RNA analysis. Groups of at least 200 staged oocytes in pools of each stage were transferred to acid guanidinium thiocyanate solution for total RNA preparation as described by Chomczynski and Sacchi [19]. Tissues other than oocytes were dissected from adult frogs, frozen immediately in liquid nitrogen and later ground while frozen and extracted as for oocytes.

For Northern-blot analysis, 15 µg total RNA from each tissue was analyzed on 1% formaldehyde/agarose gels, transferred to Nytron membranes and probed with a ^{32}P-labeled 962-bp DNA fragment corresponding to a major part of the coding region of *X.laevis* CK2α [16].

In vitro synthesis of CK2α and CK2β mRNAs. For preparation of CK2α mRNA fragment to be used as internal standard in the competitive reverse PCR, a deleted CK2α cDNA was prepared by subcloning, in an *Eco*R1 site of the vector pGEM3, a 583-bp CK2α cDNA fragment containing the sequence of nucleotides 595–1173 (which includes the carboxy-coding region and 65 nucleotides after the stop codon).

A 37-bp deletion was obtained by digestion of the vector with *Ball* followed by religation. Using primer 1 (5′-G T C C G T G T T G C T T C C C G-3′) and primer 2 (5′-A C C A G C T G G T C G T A G T T A T C-3′), a 124-bp fragment was obtained by PCR amplification of the linearized deleted cDNA (designated pGEM3$\alpha\Delta$37), whereas a fragment of 161 bp was the product obtained by a similar amplification of the unaltered CK2α cDNA cloned in the same vector.

The RNA standard for mRNA quantification was then prepared by *in vitro* synthesis using *Sma*I-linearized pGEM3$\alpha\Delta$37 and T7 RNA polymerase, as described [20]. DNA was removed with DNase Q and RNA extracted with phenol/chloroform and purified using Nuctrap columns. RNA was quantified spectrophotometrically.

The CK2β mRNA standard was prepared from CK2β cDNA in a similar way except that a deletion of 70 bp was obtained using the overlap extension method described by Ho et al. [21] as follows: CK2β cDNA was amplified using the PCR primer 3 (5′-T A C G A A T T C A A A T G A G T A G C T C G 3-′) and primer 4 (5′-C A A T A G G T A G G A G C A A T G C C A C G G T T A G T C-3′).

A second PCR product was produced using primer 5 (5′-C C C C T T A A G T C A A C G C A T G G T C T-3′) and primer 6 (5′-G G C A T T G C T C C T A C C T A T T G G T C T C T C A G A-3′).

The underlined segments correspond to CK2β cDNA and the remaining sequences to *Eco*RI sites (dots) and the overlap segments.

The overlapping products of these amplifications were mixed and a third amplification performed using the terminal primers (primers 3 and 5). The *Eco*RI-treated product was subcloned in pGEM3 and purified (designated pGEM$\beta\Delta$70). Using deleted plasmid pGEM$\beta\Delta$70, primer 7 (5′-G C A C T G G A C A T G A T A C T G G A T C T A G-3′) and primer 8 (5′-G C C A A A A T A T G C T C C A T C G G T G T G A-3′), a PCR product of 276 bp was obtained, while the undeleted CK2β cDNA cloned in the same vector gave a fragment of 346 bp. pGEM$\beta\Delta$70 was linearized and RNA synthesized and processed as described above for CK2α.

Quantitative reverse PCR. The system described by Gilliland et al. [22] was used with some changes. A constant concentration of each stage of oocyte total RNA (0.5 µg obtained from a specific number of staged oocytes) was incubated for 5 min at 70°C with each of the 14 levels of the internal standard RNA (0.01–100 pg). After cooling, the reverse-transcription reaction was carried out in a 20-µl final volume containing 0.5 mM each dNTP, 10 mM Tris/HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 20 units RNasin, 1 µM CK2α or CK2β antisense primers (corresponding to primer 2 and primer 8, respectively) and 1.2 units avian myeloblastosis virus reverse transcriptase. After incubation for 1 hour at 37°C, the PCR reaction was carried out in the same tubes in a 100-µl volume containing (final concentrations) 200 µM each dNTP, 10 mM Tris/HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton, 4 µCi [α-^{32}P]dATP, 1 unit *Taq* DNA polymerase and 0.2 µM primers 1 and 2 in the case of CK2α, or primers 7 and 8 in the case of CK2β. Samples were amplified by 30 cycles of temperature shifts at 95°C for 1 min, 52°C for 1 min and 72°C for 1 min. Products were analyzed on 1% agarose/3% Nussieve agarose gels. The gels were dried for autoradiography and scanning densitometry, then the bands were excised from gels for direct determination of radioactivity. The radioactivity present in the bands resulting from the amplification of the longer wild-type mRNA and of the shorter deleted synthetic RNA was used to determine the ratio of the two products in each determination. The concentration of the exogenous RNA at which this ratio equaled 1 defined the equivalence point.

The amount of mRNA coding for α and β CK2 subunits was calculated using the value determined from the equivalence point of the competitive PCR, correcting for the fact that these incubations were all carried out using 0.5 µg total RNA obtained from oocytes at the different stages of oogenesis. In these calculations, we used the values of total RNA content determined for *Xenopus* oocytes at different stages by Taylor and Smith [23].

Oocyte and nuclei extracts for kinase assays and Western blotting. Defolliculated oocytes prepared as described for mRNA preparation were separated into stages I–VI, chilled on ice and a specific number hand-homogenized in a 1-ml glass tube with 100 µl final volume homogenizing buffer (buffer H) containing 50 mM Tris/HCl pH 7.9, 7 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 0.02% sodium azide, 0.2 µg aprotinin, 0.4 µg each leupeptin and pepstatin and 5 mM phenylmethylsulfonyl fluoride. For Western blotting, 30 mM benzamidine was added to buffer H. This and all further manipulations were performed on ice.

The assays of kinase activity in extracts of whole oocytes were carried out by preparing homogenates of groups of oocytes. Due to their different sizes and protein content, in the case of stage-I oocytes, groups of 40 cells were used, for stage II, groups of 15 cells, and for stages III–VI, groups of 10 oocytes. Homogenates were centrifuged at 14000 rmp (Sorvall Microspin 24S rotor) for 5 min and the supernatant solutions were diluted with Buffer H, again using different dilutions for the different stages of the oocytes. Supernatant fractions of stages II, III and IV oocytes were diluted threefold while those of stages V and VI were diluted sixfold. The supernatant solution of stage I homogenates was not diluted. Kinase assays were performed in duplicate within an hour after homogenization.

Nuclei were isolated manually from oocytes ranging from stages II–VI (oocytes in stage I were too small to allow isolation of nuclei by this method) essentially as described by Ford and Gurdon [24] except that oocytes were incubated in 250 mM sucrose and 7 mM $MgCl_2$ prior to piercing the cell to allow the nuclei to emerge. Preliminary studies showed that this treatment minimized the loss of CK2 activity from nuclei. Isolated nuclei were rinsed in the same sucrose solution and groups of 15 nuclei

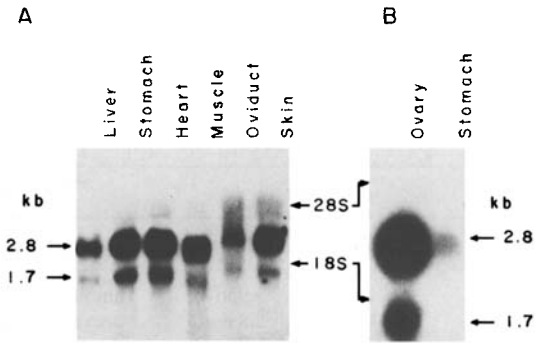

Fig. 1. Northern-blot analysis of CK2α mRNA expression in *X. laevis* tissues. Northern blots of RNAs from tissues were probed with a ^{32}P-labeled 962-bp fragment of the coding region of CK2α as described in Materials and Methods. 15 µg indicated tissue RNA was run on each lane. Positions of 28S and 18S rRNAs are shown. Exposure times were (A) 18 hours and (B) 3 hours.

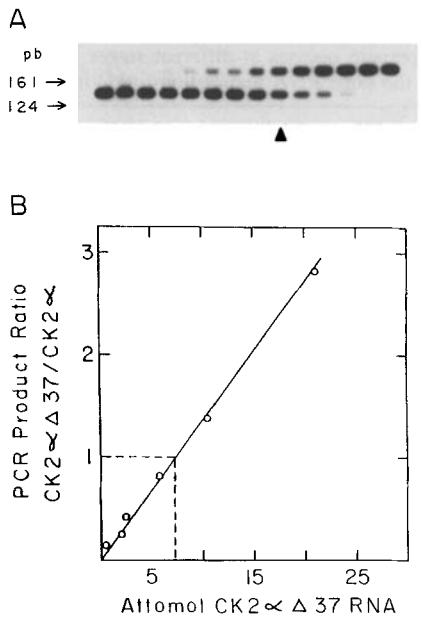

Fig. 2. Quantification of CK2α mRNA in stage-VI oocytes using competitive reverse PCR. Amplified PCR products were produced using 0.5 µg total RNA from stage-VI oocytes and different amounts of *in vitro* synthesized CK2α*Δ*37 RNA (0.01–100 pg corresponding to 0.05–525 amol) which were reverse transcribed prior to performing the standard PCR reaction in the presence of [α-^{32}P]dATP, as described in Materials and Methods. (A) Autoradiography of PCR products separated on a 1% agarose/3% Nussieve gel. Arrowhead indicates the approximate equivalence point of both products. (B) Graphic representation of the quantification of α-subunit mRNA. The bands corresponding to the amplified CK2α mRNA (161 bp) and CK2α*Δ*37 RNA (124 bp) were excised and radioactivity determined. The ratio of the radioactivity of the PCR products was plotted against the amount of input CK2α*Δ*37. The broken lines indicate the equivalence point at a ratio of 1:1.

were transferred in exactly 10 µl to the homogenizer. 100 µl buffer H was added to homogenize the nuclei and the extracts were diluted twofold (stage IV–VI) in the same buffer just prior to the kinase activity assay. Stage II and stage III nuclei homogenates were assayed without dilution.

Protein kinase CK2 assay. Oocyte and nuclear extracts were assayed essentially as described by Gatica et al. [25] in a 30-µl volume containing 50 mM Hepes, pH 7.8, 150 mM KCl, 10 mM $MgCl_2$, 10 µM dithiothreitol, 10 µM protein kinase A inhibitor peptide, 50 µM [α-^{32}P]ATP (900–1100 cpm/mol) and either 5 mg/ml β-dephosphocasein or 1 mM CK2 synthetic peptide substrate (R R R D D D S E D D). Parallel reactions were carried out in the presence of 10 µg/ml heparin. The reaction was initiated by the addition of extract and the incubation carried out for 10 min at 30°C. Aliquots were transferred to squares of Whatmann P81 paper and processed as described [25]. Assays were performed in duplicate using extract volumes which gave linear activity responses.

Radioactivity observed in the presence of heparin, a potent inhibitor of CK2, was subtracted from that observed in the absence of this inhibitor in parallel assays.

CK2 activity was expressed taking into account the number of oocytes or nuclei present in the volume of homogenate assayed.

Immunoblot analysis. Antiserum against CK2α was prepared by immunizing rabbits with the peptide I L G R H S R K R W E R F corresponding to amino acids 272–284 of the *X. laevis* CK2α sequence [16]. The peptide was coupled to bovine serum albumin and antiserum prepared in rabbits by repeated immunizations. Oocyte extracts were prepared in buffer H with 30 mM benzamidine added, using a specific number of staged oocytes, as given for activity measurements.

Protein concentrations were determined and a constant amount (20 µg) protein from each oocyte stage used for 10% SDS/polyacrylamide electrophoresis. Stained molecular-mass standards and 56 ng recombinant CK2α [17] were also run. This antibody was shown to react with the recombinant α′ subunit of CK2 from zebrafish (Antonelli, M., Daniotti, J. L., Rojo, D., Allende, C. C. and Allende, J. E., unpublished results). Transfer was made to a nitrocellulose membrane, filters blocked with 'Blot-qualified' bovine serum albumin in buffered saline (150 M NaCl, 10 mM Tris/HCl, pH 7.4) and incubated overnight at 4°C with a 1:1200 dilution of the anti CK2α serum in blocking buffer.

Filters were washed and the subsequent incubation with alkaline-phosphatase-linked second antibody was performed according to the instructions of the manufacturer (Western Blot AP system, Promega). Bands were analysed by scanning densitometry and the relative concentration of CK2α/oocyte was determined according to the number of oocytes of each stage present in the volume of homogenates analysed in the gel. Since the antiserum detected a doublet of proteins, the total density for both bands was used for this calculation. Controls were run in which the CK2α peptide serum was absorbed with 75 µM of the synthetic peptide used for immunization.

Other procedures. Autoradiography was performed using Kodak X-Omat film. Scanning densitometry was performed with a Logitech Scanman 256 and the program Gel Perfect (Bozzo). Protein concentrations were determined by the method of Bradford [26] with bovine serum albumin as a standard.

RESULTS

The amount of mRNA coding for CK2α is very high in the *Xenopus* ovary. Northern-blot analysis of the RNAs from different tissues of adult *X. laevis* (Fig. 1) gave two positive signals for all tissues examined; a strong signal indicating a mRNA of approximately 2.8 kb and a weaker signal corresponding to a mRNA of 1.7 kb. Equivalent amounts of total RNA from each tissued were loaded in each lane. The equivalence in the amount of total RNA used was corroborated by staining of the 18S and 28S rRNAs which had similar intensities (not shown). Therefore, the variations in the intensity of the signal of the probe in

Table 1. Protein kinase CK2 mRNA levels during oogenesis. mRNA coding for α and β subunits was quantified by competitive reverse PCR and the results calculated as the amount of transcripts/oocyte, as described in Materials and Methods. The ovaries of four different animals (A–D) were used to prepare oocytes at different stages. The results given for animal A correspond to determinations of both α-subunit and β-subunit mRNAs using the same RNA preparations. n.d., not determined.

Stage of oogenesis	mRNA transcript/subunit for									
	α subunit						β subunit			
	A		B		C		A		D	
	amol/ oocyte	relative increase	amol/ oocyte	relative increase	amol/ oocyte	relative increase	amol/ oocyte	relative increase	amol/ oocyte	relative increase
I+II	44	1	40	1	28	1	10.2	1	2.7	1
III	80	1.8	77	1.9	42	1.5	19.4	1.9	9.5	3.5
IV	97	2.2	85	2.1	64	2.3	22.3	2.2	21.4	7.9
V	124	2.8	105	2.6	62	2.2	31.1	3.0	26.1	9.6
VI	106	2.4	n.d.	–	58	2.1	n.d.	–	16.7	6.2

all lanes indicate differential amounts of the mRNA coding for this gene. It is noteworthy that ovary gives a much stronger signal than stomach tissue (Fig. 1 B), which in turn is one of the tissues where this mRNA is relatively abundant (Fig. 1 A).

The amounts of mRNA specific for CK2α and CK2β in different stage ooctyes were quantified by competitive reverse PCR using the technique described by Gilliland et al. [22] and also used by Oñate et al. [27] to determine the levels of mRNAs coding for gunanosine-nucleotide-binding α subunits in *X. laevis* oocytes. This procedure relies on the addition of known amounts of a deleted version of the mRNA under study to the reverse-transcriptase PCR system.

All six stages of defolliculated oocytes were examined by comparing PCR products obtained using primers from the α and β CK2 encoding sequences (Fig. 2). Plasmids with small deletions in these sequences were prepared to obtain RNA products through *in vitro* transcription for use in the competitive PCR reaction. The equivalence point at which the same amount of product is obtained with endogenous CK2$\alpha\Delta$37 mRNA added and the added deleted RNA (Fig. 2 A, indicated by the arrowhead) was estimated using densitometric analysis of the gel bands. A more precise estimation of mRNA levels is obtained by determining the ratio of the two PCR products obtained at each different level of added deleted mRNA by determining the radioactivity in each band (Fig. 2 B). The equivalence point (ratio = 1) in this case is obtained at 7.5 amol mRNA added. Similar experiments were performed with RNA isolated from the other stages of oogenesis for determination of both CK2α and CK2β mRNA levels. In total, oocytes from eight animals were analyzed.

Although the absolute values from four different representative animals (Table 1) vary considerably from animal to animal, possibly because of their seasonal and hormonal state, in all cases there is a very consistant increase in the amount of mRNA for both CK2α and CK2β. The mRNA for CK2α increases 2–3-fold as oocytes grow from stages I–II to stages IV–V. The mRNA of CK2β is present at a significantly lower concentration compared to α, but similar increments during oogenesis are also consistently observed in the animals tested. When the two mRNAs were analyzed from the same animal (Table 1; animal A), the increments of the two mRNAs were quite similar.

In order to evaluate the levels of CK2 catalytic activity present during the same periods of oogenesis, soluble extracts from whole oocytes and isolated nuclei were prepared from defolliculated oocytes. These assays were performed using a synthetic peptide substrate specific for CK2 as well as dephosphocasein.

Table 2. Protein kinase CK2 activity during oogenesis. Activity measurements were determined in duplicate, as described in Materials and Methods, in the presence of either casein or synthetic peptide substrate. The date are presented as the mean ± SEM, where n is the number of different females analyzed. n.d. not determined.

Oocyte stage	CK2 activity for			
	oocytes		nuclei	
	casein ($n=5$)	peptide ($n=2$)	casein ($n=5$)	peptide ($n=3$)
	(pmol · min^{-1} · oocyte^{-1})		(pmol · min^{-1} · nucleus^{-1})	
I	0.12 ± 0.03	0.08 ± 0.0	n.d.	n.d.
II	0.49 ± 0.12	0.36 ± 0.05	0.19 ± 0.06	0.10 ± 0.03
III	1.2 ± 0.10	1.3 ± 0.30	0.35 ± 0.06	0.24 ± 0
IV	2.5 ± 0.36	2.1 ± 0.80	0.60 ± 0.25	0.32 ± 0.05
V	3.6 ± 0.75	4.8 ± 0.70	1.1 ± 0.20	0.76 ± 0.02
VI	6.2 ± 0.49	6.8 ± 0.51	1.3 ± 0.26	0.93 ± 0.4

Since it is well known that oocyte extracts contain multiple protein kinases, particularly the cAMP-dependent protein kinase, the specific inhibitor peptide PKI was added to assays at levels which achieve maximal PKA inhibition [28]. These conditions, together with the use of control assays in the presence of heparin, an efficient inhibitor of CK2 [29], permit a reasonable estimation of the level of CK2 activity in these crude extracts.

Comparison of activities from whole oocyte extracts at stages II–VI shows an increase in CK2 activity of 12-fold (casein substrate) to 16-fold (peptide substrate), whereas CK2 activity measured in nuclear extracts increased fourfold (casein substrate) to sixfold (peptide substrate). The activity residing in the nucleus varies from 30% (stage-II nuclei) to 15% (stage-VI nuclei).

The CK2α protein levels in oocyte extracts were also analyzed by Western blotting using an antiserum prepared against a 13-amino-acid synthetic peptide designed from the CK2α cDNA sequence. This antiserum recognized the recombinant CK2α subunit from *Xenopus* and the recombinant CK2α' subunit from zebrafish that had been expressed in *E. coli* and purified (Antonelli, M., Daniotti, J. L., Rojo, D., Allende, C. C. and Allende, J. E., unpublished results). Analyzing oocyte extracts by Western blotting with this antiserum yielded two prominent bands corresponding to proteins of 43 kDa and 40 kDa, the approximate

values of CK2α and CK2α' reported for several species (not shown). Both of these positive signals were eliminated by absorption of the antibody with the synthetic peptide used to generate the antiserum. However, in these blots of whole extracts, some unspecific bands were also present. Densitometric quantification of the 43-kDa and 40-kDa Western-blot signals in gels containing the same amount of protein of extracts from oocytes at different stages of oogenesis indicated that the combined amount of the two CK2α-like bands increased approximately 10-fold from stage I to stage VI. This increase roughly paralleled the increment in CK2 activity.

DISCUSSION

Northern-blot analysis of different tissues of *X. laevis* indicate that the ovary of this species is unusually rich in the mRNA coding for the α subunit of protein kinase CK2. This observation induced us to study the expression of the α and β subunits in the oocytes of this species present in the ovary at different stages of oogenesis.

The competitive reverse-PCR technique is very useful to determine absolute concentrations of mRNAs because the addition of a known amount of an analogous and deleted mRNA fragment can serve both as a control and as an internal standard for this determination [22]. Using this method, results (Table 1) indicate that the amount of mRNA for both CK2 subunits increases significantly in different animals tested; 2.5-fold for the α subunit and threefold or more for the β subunit in the more advanced stages of oogenesis (stage II to stages V and VI).

This observation is interesting because it indicates that the mRNAs coding for these subunits of CK2 behave differently from the majority of the other poly(A)-containing mRNAs that are coded by the oocyte nuclear genome. Golden et al. [11] conducted an extensive survey of mRNAs present in *X. laevis* oocytes and in tadpoles and found that the vast majority of the nuclear-coded mRNAs studied ceased to increase during oogenesis after stage II. These researchers examined 500 different random clones and estimated that, if increases of twofold or more had occurred, they would have been detected by their method. This same technique was able to detect large increases in the mRNAs encoded by mitochondrial DNA.

More recently, Oñate et al. [27], also using the competitive reverse-PCR method, did not find any increases after stage II of oogenesis in the mRNA coding for the α subunit of the Go GTP-binding protein involved in signal transduction. Kobayashi et al. [30] also reported detection of constant levels of mRNAs coding for the A-type and B-type cyclins after the previtellogenic stages of oogenesis. Similarly, no significant changes in the c-raf [31] and c-rel [32] mRNAs have been detected during oogenesis after stage II is reached. Rebagliati et al. [33], who studied mRNAs which were specifically located either in the animal or vegetal pole of the *X. laevis* oocytes, found that the amounts of certain of these RNAs steadily increased throughout oogenesis, reaching a maximum in full-grown stage-VI oocytes. Also, studies carried out to determine the content of the mRNA coding for the *X. laevis* ras protein [13] detected a gradual fourfold increase in content in progressing from stage-I to stage-VI oocytes.

It must be stated, however, that the increments in the content of mRNAs coding for the CK2 subunits from stages I–II to stage VI found in this study are modest when compared to the increase of total RNA through oogenesis, which has been calculated by Taylor and Smith [23] to be about 25-fold. This increase, however, is almost totally due to ribosomal RNA synthesis since, after stage III, the oocytes use their greatly amplified nucleoli to transcribe the rRNA genes [10].

Another important aspect to be considered in relation to the results obtained in this study is the number of molecules of CK2α and CK2β RNAs found in oocytes. In the full-grown stage-VI oocytes, there are approximately 5×10^7 molecules CK2α mRNA and 1×10^7 of CK2β mRNA. The amount of CK2α mRNA is comparatively high since it is similar to the amounts of oocyte cyclin mRNAs [30], threefold the amount of ras mRNA [13], and 100-fold greater than the amounts calculated for the Go subunit of the GTP-binding protein [27] and c-rel [32]. This unusually high number of molecules of CK2α mRNA in the oocytes is about 25% the amount of mRNA coding for the very abundant histone varieties [34].

It is difficult to speculate why the mRNAs coding for CK2 subunits increase during oogenesis and why this mRNA is relatively plentiful in these cells. However, it is interesting to note that the CK2 protein has been found to be particularly concentrated in nucleoli [35] and has been postulated to play a role in ribosomal RNA synthesis [36], a process that is highly active in the midstage of oogenesis [10].

The coordinate increase in the mRNA content for both subunits observed throughout oogenesis is also interesting since it suggests that some common factors may regulate the expression of both genes.

The measurements of the CK2 catalytic activity using either heparin-sensitive casein phosphorylation or the phosphorylation of a model peptide specific for CK2 [37], also demonstrated a substantial increase during oogenesis. These activity levels increase 12–15-fold from stage II to stage V. Previous results from this laboratory have demonstrated that the activity of another enzyme, adenylyl cyclase, which is located in the plasma membrane, increased approximately 10-fold during oogenesis [38].

There has been some controversy regarding whether CK2 is or is not a predominantly nuclear enzyme [1, 39]. There are convincing recent reports which indicate that the proportion of CK2 in the nucleus varies with the growth stage of the cells in question and that signals that induce cell division cause translocation of CK2 from the cytoplasm to the nucleus [40, 41]. In the present study, using nondividing oocytes, it is found that 65–80% of the CK2 activity is not in the nucleus. However, since the nucleus is approximately 10% of the total available (non-yolk) volume of the oocyte, CK2 enzyme concentration is higher in the nucleus than in the rest of the cell. The results obtained with the antiserum prepared against a peptide sequence present in CK2α corroborate the results obtained by measuring enzyme activity. This antiserum specifically detects two protein bands that migrate at 43 kDa and 40 kDa and that seem to correspond to the α and α' species. The combined amount of these two proteins/oocyte, as detected by densitometry of the Western blots, increases about 10-fold as oogenesis progresses from stage-I to stage-VI cells. These studies, however, should be complemented by experiments using specific antibodies against the α' subunit and with anti-(β-subunit) serum. In this regard, it will be particularly interesting to elucidate whether the excess of mRNA coding for the α subunit with respect to the β subunit observed in oocytes is also reflected at the protein level, and whether or not this differential expression has any physiological meaning.

Oogenesis prepares the germ cell for fertilization and embryo development. In amphibians, oocytes contain the bulk of the constituents that are going to be needed by the embryo during its early stages. Components such as ribosomes, histones and maternal mRNAs and tRNAs that are used during the explosive period of rapid cell division and high biosynthetic activity of the early embryo are synthesized and stored during oogenesis. It is possible that CK2 may also be required at high levels during

that embryonic period, and for that reason its expression is especially important during oogenesis.

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