FEBS 22985 FEBS Letters 462 (1999) 353–357

Protein kinase CK2: evidence for a protein kinase CK2β subunit fraction, devoid of the catalytic CK2α subunit, in mouse brain and testicles

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Received 11 October 1999; received in revised form 4 November 1999

Edited by Shmuel Shaltiel

Abstract The highest CK2 activity was found in mouse testicles and brain, followed by spleen, liver, lung, kidney and heart. The activity values were directly correlated with the protein expression level of the CK2 subunits α (catalytic) and β (regulatory). The α′ subunit was only detected in brain and testicles. By contrast, Northern blot analyses of the CK2α mRNA revealed a somewhat different picture. Here, the strongest signals were obtained for brain, liver, heart and lung. In kidney, spleen and testicles mRNAs were only weakly detectable. For CK2α′ mRNA distribution strong signals were observed for lung, liver and testicles. In the case of CK2β mRNA the highest signals were found for testicles, kidney, brain and liver. The amount of CK2β mRNA in testicles was estimated to be about 6-fold higher than in brain. The strongest CK2β signals in the Western blot were found for testicles and brain. The amount of CK2β protein in brain in comparison to the other organs (except testicles) was estimated to be ca. 2–3-fold higher whereas the ratio of CK2β between testicles and brain was estimated to be 3–4-fold. Results from the immunoprecipitation experiments support the notion for the existence of free CK2β population and/or CK2β in complex with other protein(s) present in brain and testicles. In all other mouse organs investigated, i.e. heart, lung, liver, kidney and spleen, no comparable amount of free CK2β was observed. This is the first physiological evidence for the existence of a 'free CK2β' (or in complex with proteins other than CK2α) in normal animal tissue apart from the hitherto dogmatic association with CK2α in a tetrameric holoenzyme complex.

Key words: Protein kinase CK2; Free CK2β; Mouse organ

1. Introduction

Protein kinase CK2 is a pleiotropic and ubiquitous protein kinase found from yeast to man, present in all so far investigated tissues and organs. In mammalian organisms, two catalytic subunits, i.e. α and α′ and a regulatory CK2β subunit were identified. The enzyme can exist as $\alpha\alpha'\beta_2$, $\alpha'_2\beta_2$ and $\alpha_2\beta_2$. All three subunits are located on different chromosomes [1–3]. The functional difference of the two different catalytic subunits is unknown as is the physiological role of the holoenzyme. Evidence for an important role however, is intriguing by observations such as elevated expression in all cancers so far investigated, induction of lymphocyte transformation in transgenic mice and requirement for a variety of viruses to propagate (for review see [4]). Taken all these data into consideration one may hypothesize that CK2 plays an important role in proliferation and transformation [4,5]. But beside these observations more and more data are amassing suggesting a pivotal role for the CK2 subunits themselves. The first evidence came from Stigare et al. [6] who showed by sequential extraction methods that the individual subunits could be selectively extracted from the chromatin. Stalter et al. [7] showed that in renal clear cell carcinomas there is evidence for excess of CK2β subunits. Chen et al. [8] showed that CK2β subunit binds to Mos and inhibits Mos activity and thereby inhibits meiotic maturation during oocyte development.

Studies by Lüscher and Litchfield [9] using a lymphoid cell line showed that the CK2β subunit was synthesized in excess over the catalytic subunit and that a substantial fraction of the newly synthesized CK2β was degraded within the first hour.

It is easily imaginable that a scenario preventing the degradation would lead to an imbalance of β/α giving way for a CK2β role different from that it has in the holoenzyme.

Further evidence for a different role of CK2β was obtained by yeast two-hybrid studies. CK2β-specific interacting partners were identified, e.g. A-Raf, L5, FAF-1 [10]. Interestingly, some of these proteins were identified separately by other researchers using the CK2β-specific interacting partners as search tools.

In the case of L5 the yeast two-hybrid data were confirmed in vivo by immunoprecipitation studies involving CK2-specific antibodies [11]. Despite these encouraging findings we are still lacking more supportive evidence for the presence of CK2 subunits other than in complex with the holoenzyme. A systematic analysis of selected mouse organs with respect to the quantitative distribution of the three CK2 subunits revealed now that there is more CK2β in brain and testicles than necessary for holoenzyme complex formation. This is the first evidence in normal mammalian organ material for the existence of free CK2β or in complex with other protein(s), but devoid of CK2α.

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PII: S0014-5793(99)01553-7

2. Materials and methods

2.1. Mouse organ extract preparation

Crude extracts were prepared by homogenization with an Ultraturrax of the minced organ material in 25 mM Tris/HCl, 1 mM DTT, 200 mM NaCl, pH 8.5 in the presence of protease inhibitors (Roche). Subsequently the homogenate was sonicated and centrifuged at 4°C, 30 min at 4000×*g*. The supernatant was collected and the protein concentration was determined by the method of Bradford using bovine serum albumin as a standard [12].

2.2. Protein kinase assay

Crude extracts from mouse organs were assayed for protein kinase activity at 37°C for 5 min using the synthetic peptide RRRDDDSDDD as a substrate [13,14]. One U of CK2 is defined as the amount of activity necessary to transfer 1 pmol phosphate/min into the synthetic peptide substrate at 37°C. Each value shown in Table 1 is the result of three independent determinations.

2.3. SDS-polyacrylamide gel electrophoresis and Western blot analysis

Detection of the individual proteins by Western blotting analysis was carried out by incubating the membranes with different CK2 antibodies. (i) The polyclonal antibodies directed against the three human CK2 subunits were all raised in rabbits and used at a dilution of 1:100. The rabbits were immunized with purified recombinant CK2α and CK2β subunits. Only in the case of the CK2α′ a specific peptide sequence was used for immunization, i.e. SQPCANAVLSSGTAAR. (ii) The monoclonal antibodies 1AD9 (anti-CK2α) and 6D5 (anti-CK2β), were purchased from Calbiochem. When not otherwise indicated 12.5% SDS-PAGE gels were used for the analysis of mouse organ extracts. In all lanes the same amount of protein was loaded. In cases where the stained proteins did not match, adjustments were made. Hence, the signal intensities of the individual subunits in the different organs can be compared. The proteins were blotted to a PVDF membrane. After the transfer the membrane was cut in two pieces horizontally according to the molecular mass as indicated by the colored rainbowmarker. The upper piece was used for the detection of the α subunit, the lower piece for the detection of the CK2β subunit. The CK2α′ was detected on a separate membrane. The CK2β was detected with an affinity-purified polyclonal antibody. All the β subunits in the different organs investigated are detected with the same antibody dilution and hence the amounts detected can be compared with each other. The α/α′ subunits were detected by using affinity-purified polyclonal antibodies. Again as already done in the case of the CK2β, the organ material can be compared with each other with respect to the corresponding subunit. However, not between each other. A comparison of the three subunits, with respect to their expression level from one organ is not possible owing to the different antibody specificities and avidities.

2.4. cDNA probes

cDNA probes for the murine CK2α and CK2β were obtained by PCR using 10 ng plasmid DNA as template and 20 pmol of each, an upstream and downstream primer in a 100 μl reaction containing 200 μM dNTPs, 10 μl 10×Taq buffer and 2.5 U Taq polymerase (Pharmacia). For CK2 an RT-PCR reaction using RNA isolated from mouse testicles was performed with the GeneAmp RNA PCR kit from Perkin Elmer. Primer for all PCR reactions was selected in a way that the corresponding full length coding region was amplified. PCR products were purified with the Qiaquick gel extraction kit (Qiagen). One hundred ng of purified PCR product was labeled with a DNA labeling kit (MBI Fermentas). For hybridization the Quikhyb hybridization solution (Stratagene) was used.

2.5. RNA extraction and detection

Total RNA was isolated by use of TRIZOL-Reagent (Life Technologies) following the protocol of the supplier. All RNA samples had an $A_{260/280}$ ratio between 1.8 and 2.0. After running a match gel, 18 μg of total RNA was separated on a formaldehyde gel (0.8% agarose) with ethidium bromide directly added to the loading buffer. The transfer was carried out by capillary transfer overnight. The nitrocellulose membrane was baked at 80°C for 1 h. The prehybridization was done at 68°C for 20 min in QuikHyb solution (Stratagene). For hybridization solution we used the prehybridization solution plus labeled DNA of approximately 1.8×10^6 cpm/ml. Hybridization was performed for 1 h at 68°C. The washing was carried out with 2×SSC (0.3 M NaCl, 0.03 M Tri-sodiumcitrate) and 0.1% SDS twice for 15 min at room temperature and once for 30 min at 60°C with 0.1×SSC and 0.1% SDS. Autoradiographs were exposed to X-ray film (X-Omat AR, Kodak) at −80°C with an intensifying screen for 1–3 days.

2.6. Immunoprecipitation

400 μg crude extract from either brain or testicles were used in a volume of 300 μl. The volume was adjusted with NET-modified buffer: 150 mM NaCl, 5 mM EDTA, 50 mM Tris/HCl, 0.05% NP40, 0.02% NaN_3, 0.2% casein, pH 8, and inhibitor cocktail (Roche). Incubation was overnight at 4°C. The following immunoprecipitations were carried out: (1) brain extract+4 μl pre-immune serum, (2) brain extract+4 μl polyclonal anti-CK2α serum+4 μl polyclonal anti-CK2α′ serum, (3) testicles extract+4 μl polyclonal anti-CK2α serum+4 μl polyclonal anti-CK2α′ serum. After the incubation the resin was spun down and the supernatant dialyzed overnight against double distilled (d.d.) water. The samples were then lyophilized and resuspended in SDS sample buffer. Eighty μg of each sample were loaded on a gel followed by Western blotting and immunostaining with monoclonal anti-CK2α antibody (1AD9, 0.5 μg/ml) and with monoclonal anti-CK2β antibody (6D5, 0.7 μg/ml).

3. Results and discussion

3.1. Northern analysis

3.1.1. CK2α. Total RNA was isolated from the organs and then analyzed on an agarose gel, blotted to a nitrocellulose membrane and probed with a CK2α-specific cDNA. Fig. 1 shows the Northern blot analysis. As reported earlier three major signals of 4.8, 3.2 and 2.0 kb were detected [15]. The overall signal intensity was strongest in brain and heart. In liver and lung the 4.8 kb band was abundant whereas in kidney and spleen the overall signal intensity detected was extremely low, a result which came as a surprise, especially with respect to the testicles. We have no profound explanation for this.

3.1.2. CK2α′. Two major signals were obtained in the case of the CK2α′ hybridization. The bands were detected

Table 1
Comparative analysis of protein kinase CK2 activity values from different mouse and rat organs

	This paper pmol/min/mg RRRDDDSDDD	Diaz-Nido et al. [18] pmol/min/mg RRREEETEEE	Hei et al. [19] pmol/min/ml phosvitin	Yutani et al. [20] nmol/nuclei cont. 1 mg DNA phosvitin
Brain	992 (66.5)	352 (99)	30 (54.5)	3.12 (66.6)
Heart	216 (14.5)	40 (14)	–	2.56 (55.6)
Lung	268 (18)	72 (25)	–	–
Liver	338 (22.6)	50 (17.4)	–	3.06 (66.4)
Kidney	239 (16)	83 (28.9)	–	1.86 (40.3)
Testicles	1492 (100)[a]	356 (100)[a]	50 (91)	4.68 (100)[a]
Spleen	674 (45)	–	55 (100)[a]	1.86 (40.3)

[a]The numbers in brackets indicate the percent of activity, as normalized to the highest activity measured.

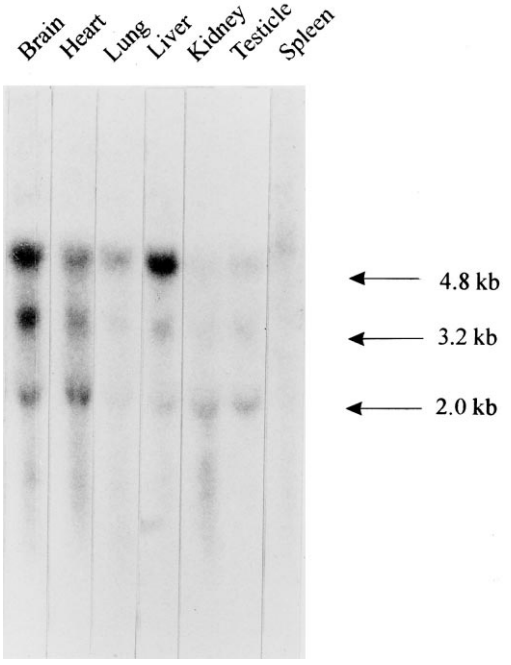

Fig. 1. Northern blot analysis of CK2α in different mouse organs as indicated in the figure. For further details refer to Section 2. The size of the major mRNA species detected by the radioactive CK2α cDNA probe is indicated by the arrows.

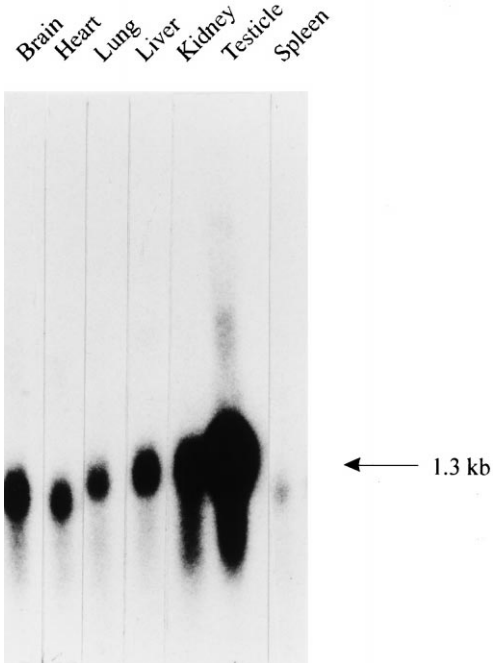

Fig. 3. Northern blot analysis of CK2β in different mouse organs as indicated in the figure. For further details refer to Section 2. The size of the major mRNA species detected by the radioactive CK2β cDNA probe is indicated by the arrows.

at 4.3 and 1.9 kb, respectively. Here, the strongest signals for the two bands were obtained for testicles and lung (Fig. 2). In the case of liver, kidney and brain predominantly the 1.9 kb band hybridized with the CK2α′-specific probe. We cannot assess whether the observed differences in the hybridization pattern are of any significance, since we do not know the importance of the two mRNA species in the first place.

Other authors [16,17] have already speculated earlier that the two isozymes may serve different functions. One reflection of functional specialization might be tissue- or cell-type-specific.

Quantitation of the results obtained in different adult chicken tissues shows that CK2 mRNAs are considerably more abundant in certain tissues (e.g. spleen, brain, ovary and heart) than in others (e.g. kidney and lung). Spleen and heart express high levels of CK2α but low levels of CK2α′ mRNA whereas the opposite is true for liver, brain, and ovary.

3.1.3. CK2β subunit. The results obtained from the hybridization of the CK2β cDNA probe show a very strong signal of 1.3 kb in the case of the mRNA and equal strong signals in the brain and kidney followed by weaker signals in liver, heart and lung. The signal obtained from spleen mRNA was the weakest (Fig. 3). The signal in the case of the testicles is about 6-fold higher than the corresponding CK2β mRNA signal found in brain. It looks as if CK2β mRNA expression is not generally balanced to match the sum of the α and α′ mRNAs. This result was also obtained by [15,16] in the case of chicken tissues.

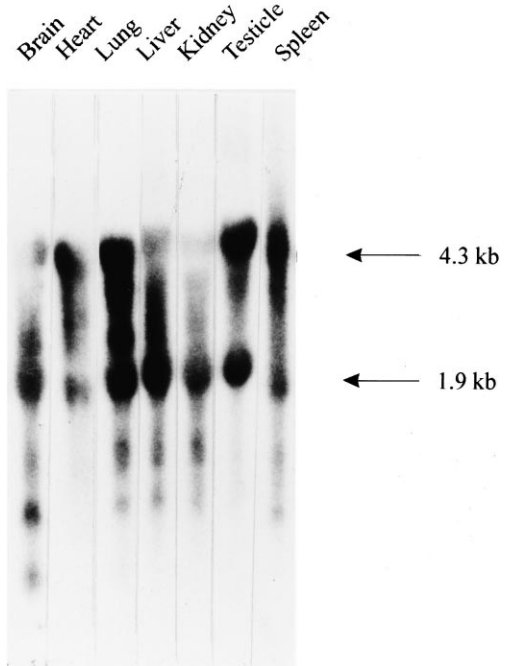

Fig. 2. Northern blot analysis of CK2α′ in different mouse organs as indicated in the figure. For further details refer to Section 2. The size of the major mRNA species detected by the radioactive CK2α′ cDNA probe is indicated by the arrows.

3.2. Protein kinase activity

In the present report, the protein kinase CK2 activity is determined in seven different murine organs, i.e. brain, heart, lung, liver, kidney, testicles and spleen. Table 1 shows the activity values obtained. The organs with the highest protein kinase activities are brain and testicles followed by spleen with about half the activity. The other organs, i.e. heart, lung, liver and kidney show only a fifth to a seventh of the activities found in brain and testicles, respectively. A comparison with previous measurements in rat yielded a similar distribution

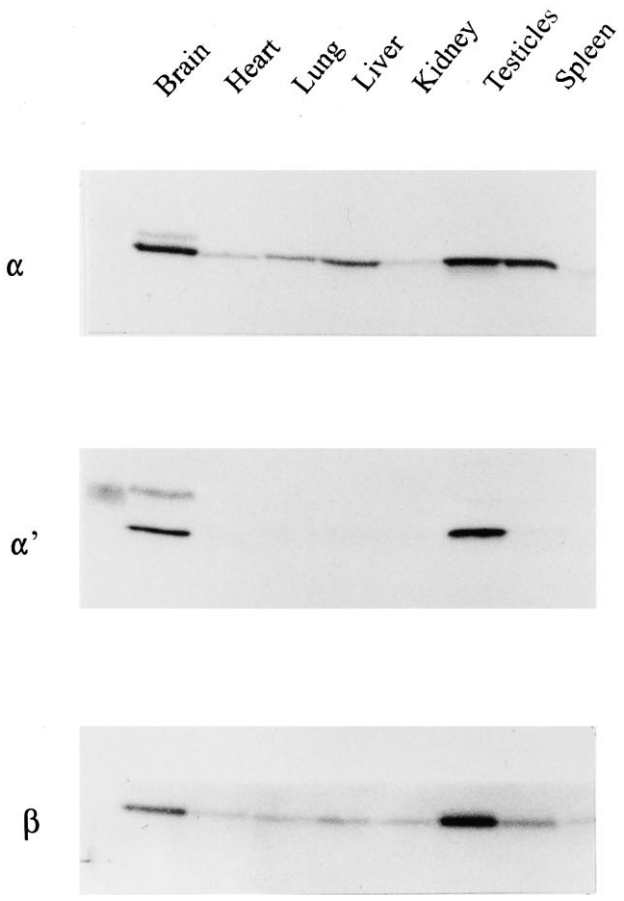

Fig. 4. Western blot analysis of CK2 subunits distribution in different mouse organs as indicated in the figure. Detection of CK2α, α′, and β subunits is shown sequentially from top to bottom.

[18]. These authors also found the highest CK2 activities in brain and in testicles. Hei et al. [19] by contrast, found most of the murine CK2 activity in spleen, testicles and brain. The discrepancy to our results may be in part explained by the fact that the latter authors did not use the synthetic peptide but rather phosvitin as a substrate. Since phosvitin can be phosphorylated also by other protein kinases, foremost by CK1, a different activity distribution does not come as a surprise. A paper from Yutani et al. [20] investigating CK2 activities in nuclei of various tissues showed the highest activity to be present in testicles, brain and liver.

Fully in agreement with our results are the data obtained from Diaz-Nido et al. [18]. The highest CK2 activities (100%) were found in testicles, followed by brain in mouse (66.5%) and in rat (99%). This represents a slight discrepancy similar like the value for kidney where in rat ca. 29% were found as compared to 16% in mouse. Heart, lung and liver CK2 activity values are in good agreement. The overall low activity values in the case of rat do not come as a surprise. Diaz-Nido et al. [18] used a synthetic peptide which has a threonine as a phosphoryl acceptor whereas we used a serine, the latter being a much better acceptor.

3.3. Western blot analyses

A comparison of the individual CK2 subunits present in the different organs was carried out by Western blotting and subsequent immunostaining with specific antibodies. Fig. 4 shows an immunoblot of the three subunits investigated, i.e. CK2α, α′ and β. All three subunits can be detected albeit with different intensities, reflecting the CK2 activity values.

The CK2α subunit detection is strongest in testicles followed by brain and spleen, liver and lung. The lowest amounts are detected in heart and kidney. This is exactly what is also found by activity measurements (Table 1). Here too, we find the highest activity in the testicles, followed by brain and spleen and the lowest activities in liver, lung, heart and kidney.

The CK2α′ subunit was only detected in testicles and brain. We will not exclude the presence of this subunit also in other organs but under standard detection conditions using the highly sensitive dioxetane chemoluminescence agent (Tropix) no CK2α′ subunit was found in the other organs. In testicles we find ca. twice as much CK2α′ subunit than in brain.

In agreement with the CK2α distribution the CK2β subunit is also detected in all organs. It is strongest in testicles and in brain followed by spleen. In the case of the other organs the intensities of the CK2β signals are in good agreement with the intensities seen in the case of the CK2α subunit.

The signal intensity of the CK2β subunit in testicles is estimated to be about 3–4-fold higher than what was found in the brain and more than 8–10-fold higher than in the other organs (Fig. 4).

If one compares the CK2α signals in testicles and brain there is only a slightly higher expression in testicles and as already pointed out the difference between the CK2α′ between testicles and brain is about 2-fold. Hence, there is a significant higher amount of CK2β present in the testicles (Fig. 4). This finding is intriguing since it supports the notion of a CK2β subunit not in association with the CK2α and α′ subunits, because these subunits are present in almost comparable ratios. We would like to emphasize that the immunoblots shown in Fig. 4 allow only a horizontal comparison. A comparison of the intensities of the individual subunits from one organ is prohibitive because of the different antibody specificities and avidities.

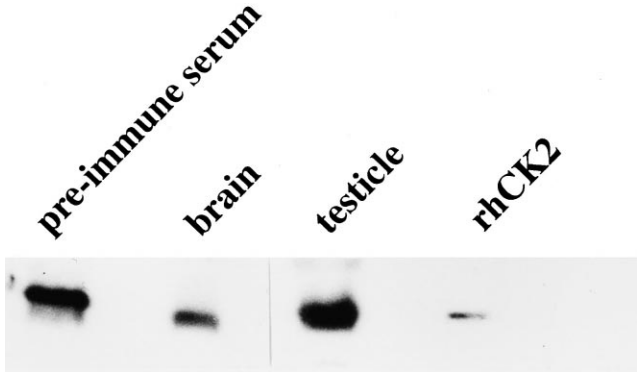

Fig. 5. Immunoblot showing free CK2β subunit present in the supernatant after immunoprecipitation of brain (lane 2) and testicles (lane 3) crude extracts with polyclonal antibodies directed against the human CK2α and CK2α′ subunits. Lane 1 (control) shows the detection of the CK2β subunit in the supernatant of brain crude extract after immunoprecipitation with normal rabbit serum (pre-immune serum). Lane 4 (control) shows the detection of CK2β in the case of the recombinant CK2 holoenzyme (30 ng).

The CK2β mRNA levels from brain and testicles (Fig. 3) match those found for CK2β protein in these two organs (Fig. 4). This finding, although suggestive, may still be coincidental because it is known from previous work on the distribution of the CK2 mRNA in chicken and Drosophila that the amount of mRNA detected did not reflect the observed protein levels [15,16] and the same is true for some of our data involving the CK2α mRNA expression levels in testicles (Fig. 1). The difference in the expression level of the CK2β protein between testicles and brain is about 3–4-fold whereas the difference at the mRNA level is about 6–8-fold, i.e. ca. twice as high. Whether this large amount of CK2β mRNA is subject to a specific transcriptional control in testicles is subject of further investigations. Furthermore, we would like to explore the function of the CK2α′ in testicles and brain, especially with respect to the significant higher protein kinase activity in these two organs.

3.4. Immunoprecipitation studies

We have carried out immunoprecipitations using testicles and brain extracts. The extracts were incubated with a mixture of polyclonal anti-CK2α/α′ antibodies in order to precipitate foremost CK2 holoenzyme. The supernatant was checked for the presence of remaining CK2α/α′. Except for the pre-immune serum no α/α′ subunit was detected (results not shown). The obtained supernatants were then further analyzed for the presence of CK2β not associated with the holoenzyme. As we can see in Fig. 5 from left to right, when we incubated the membrane with an antibody directed against CK2β we found a strong signal in the case of the brain extract incubated with the pre-immune serum (lane 1). This result was expected since the pre-immune serum should not precipitate any CK2 subunits, hence one would expect to detect all CK2β present in the mouse cell extract. There is also CK2β detectable in the brain and testicles from the IP supernatants (Fig. 5, lanes 2, 3). When other organ extracts were used for the immunoprecipitation in parallel with the brain and testicles extracts using anti-CK2α/α′ antibodies no CK2β was detected (results not shown). However, this does not exclude that there are no small amounts of free CK2β molecules still there; we only cannot detect them under the present detection conditions. Since the analyzed supernatants shown in Fig. 5 derived from extracts where the holoenzyme was removed by immunoprecipitation with anti-CK2α/α′ antibodies the presence of CK2β in the supernatants strongly suggests that there is also a population of CK2β subunits not in association with the catalytic CK2α/α′. As already mentioned earlier in this paper Chen et al. [8] found CK2β in association with Mos in maturating oocytes. Here it looks as if there is more than one particular function for the CK2β because of the evidence obtained by the immunoprecipitation studies. Whereas in the case of the testicles one could implicate a role for the CK2β in spermatogenesis the possible role in brain is totally open for speculation. Further experiments are needed in order to explore the novel new potential tasks of CK2β in normal cellular development and regulation.

Acknowledgements: This work was supported by a Grant from the Danish Cancer Society, 96 100 40 to O.-G.I.

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