Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 307 (2003) 86–91
BBRC
www.elsevier.com/locate/ybbrc

The protein of a new gene, Tctex4, interacts with protein kinase CK2β subunit and is highly expressed in mouse testis☆

Xiyuan Bai,[a,b,*] Xin Xu,[a] Fengying Zhang,[a,b] Hong-Bing Shu,[b] and Edward D. Chan[b]

[a] *McLaughlin Research Institute for Biomedical Sciences, Great Falls, MT 59405, USA*
[b] *Department of Medicine, National Jewish Medical and Research Center, 1400 Jackson St, Denver, CO 80206-2761, USA*

Received 23 May 2003

Abstract

Casein kinase 2 (CK2) is a ubiquitous, multifunctional eukaryotic serine/threonine kinase that phosphorylates an array of proteins. CK2 is a heterotetramer composed of two catalytic (α, α') and two regulatory (β) subunits. CK2 plays an essential role in regulatory pathways in cell transformation and proliferation. But the role and function of the individual subunits of CK2, which are not in the holoenzyme, are not yet clear. Northern blot analysis reveals the highest CK2β activity in mouse testicles and brain. By employing a yeast two-hybrid screen to identify the proteins that interact with CK2β, we have isolated a cDNA clone encoding a 14-kDa protein with homology to dynein light chains and have designated it as Tctex4. CK2β interacts specifically with Tctex4 both in a yeast two-hybrid system and in an in vitro interaction assay. Northern blot and in situ hybridization showed that Tctex4 is a novel gene that is expressed in mouse testis.

Keywords: Protein kinase CK2; Tctex4; Protein–protein interaction; Testis

Casein kinase 2 (CK2) is a eukaryotic serine–threonine protein kinase which phosphorylates a variety of nuclear and cytosolic proteins [1]. CK2 is well conserved, found in yeast to human, and present in a wide range of tissues and organs. In mammalian organisms, the native enzyme is tetrameric with a molecular mass of 130 kDa and composed of two catalytic subunits (α and α') and two regulatory subunits β which associate to form $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$ structures. All three subunits are located on different chromosomes [2,3]. The functional difference in the two α and α' catalytic subunits is unknown, as is the physiological role of the holoenzyme. But all these data take into consideration the fact that CK2 plays an important role in proliferation and transformation [4,5]. However, there is increasing evidence which suggests a pivotal role for CK2 subunits themselves. Targeted disruption of the CK2α' subunit in mice revealed an indispensable role for this subunit in male germ cells where it is preferentially expressed [6]. Genetic disruption of CK2α' resulted in a shape change of the murine sperm head in mice during spermatogenesis and in male infertility. Stalter et al. [7] showed that in renal clear cell carcinomas, there is increased expression 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.

In the past few years, further evidence for a different role of CK2β was obtained in yeast two-hybrid studies. CK2β-specific interacting partners were identified, e.g., A-Raf, L5, and FAF-1 [9]. Interestingly, a systematic analysis of selected mouse organs with respect to the quantitative distribution of the three CK2 subunits now revealed that there is more CK2β in brain and testicles than that necessary for holoenzyme complex formation [14]. To explore the role of CK2 in spermatogenesis, we used the CK2β-specific interacting partners as research tools. Here, we report studies aimed at identification of

☆ *Abbreviations:* CK2, casein kinase 2; GAL4-DB, GAL4 DNA binding domain; GAL4-AD, GAL4 transactivation domain; GST, glutathione-*S*-transferase; TcTex4, t-complex testis expressed 4.

* Corresponding author. Fax: 1-303-398-1806.
E-mail address: baix@njc.org (X. Bai).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)01118-5

CK2β-interacting protein from mouse testis in vivo using a yeast two-hybrid system. A cDNA clone encoding a 14-kDa protein with homology to dynein light chains gene has been isolated and designated as Tctex4. CK2β interacts specifically with Tctex4 both in a yeast two-hybrid system and in in vitro interaction assay. Northern blot and in situ hybridization demonstrated that Tctex4 is a unique gene expressed in mouse testis.

Materials and methods

Materials. Nucleotide oligomers used as polymerase chain reaction primers were synthesized by Sigma. BL-21(DE3) and BL-21(DE3) pLysS competent cells were purchased from Novagen. Yeast strain *Saccharomyces cerevisiae* Y190 (MATa, ura3-52, his3-200, ade2-101, lys2-801, trp1-901, leu2-3,112, gal4Δ, gal80Δ, cyhr2, LYS2::GAL1uas-HIS3TATA-HIS3, and URA3::GAL1uab-GAL1TATA-lacZ), pGBKT7, pACT2, and murine testis cDNA library constructed in λgt11 from mouse testis tissues were from Clontech. Restriction endonucleases and other cloning reagents were purchased from Promega (Madison, WI) and New England Biolabs (Beverly, MA). [α-^{32}P]dCTP (3000 Ci/mmol), [γ-^{32}P]ATP (3000 Ci/mmol), and L-[^{35}S]methionine (1175.0 Ci/mmol) were purchased from Perkin–Elmer Life Sciences (Boston, MA).

Construction of plasmids for two-hybrid experiments. The open reading frames (ORFs) of CK2α′, CK2α, and N- and C-terminal region of CK2β were cloned into the Gal4 DNA-binding domain vector pGBKT7 using PCR amplification and *Eco*RI/*Xho*I digestion. The DNA sequences of the fusion regions of all two-hybrid plasmid constructions were determined in order to confirm that all plasmids encoded in-frame fusions. The ORF of Tctex4 was cloned into the Gal4 transcriptional-activation domain vector pACT2.

Yeast two-hybrid screen and cDNA isolation. The full-length murine CK2β cDNA was cloned into the pGBKT7 vector in the in-frame fusion of CK2β with the DNA-binding domain of the yeast GAL4 protein. A mouse testis cDNA library containing 4.5×10^6 independent clones (Clontech) was cloned into pACT2 to produce fusions between the encoded proteins and the cDNA activation domain of GLA4. Screening was performed by sequential transformation of bait, and library vectors in *S. cerevisiae* strain Y190 used for the screening assay contained both *His*3 and *lac*Z reporter genes under the control of a GAL4-responsive upstream activation site. Transformants were plated on SC–Leu–Trp–Ade–His medium and incubated at 30 °C for up to 3–5 days. From 2 to 10^6 transformants of mouse testis cDNA in pACT2 and CK2β as bait in pGBKT7 clones were obtained a cDNA for the Tctex4 gene, a testis cDNA library in λgt11 was screened, and 8 clones were selected. The DNA sequencing was done in cooperation with Yale University. The GenBank database was searched by using the BLAST program (National Center for Biotechnology Information, Bethesda, MD).

β-Galactosidase filter and liquid assay. For the filter assay single colonies were picked and transferred to a Whatman No. 5 filter paper, which was further incubated on a fresh plate for 2–3 days. The filters were frozen in liquid nitrogen and then layered over a second filter prewetted with Z-buffer (16.1 g/L $Na_2HPO_4 \cdot 7H_2O$, 5.5 g/L $NaH_2PO_4 \cdot H_2O$, 0.75 g/L KCl, and 0.246 g/L $MgSO_4 \cdot 7H_2O$) which contained 0.27 ml of 2-mercaptoethanol and 1.67 ml X-gal (20 mg/ml in dimethylformamide) per 100 ml. Incubation was done at 30 °C for up to 4–12 h. For the liquid assay, 5-ml cultures with synthetic medium were inoculated with single colonies and grown until $A_{600} = 1.0$–1.5. Then 1.5 ml of each culture was transferred to a microcentrifuge tube and centrifuged for 30 s. The yeast pellet was dissolved in 100 μl Z buffer and frozen in liquid nitrogen. After thawing, 700 μl Z buffer with β-mercaptoethanol and 160 μl ONPG (4 mg/ml in Z buffer, freshly prepared) were added and the reaction was incubated for 30 min–1 h at 30 °C. The reaction was stopped by the addition of 400 μl of 1 M Na_2CO_3 mix, and the A_{420} was measured. β-Galactosidase activity was calculated in Miller units according to the following formula: units $= 1000 \times A_{420}$ (culture volume in ml × incubation time in min × A_{600}).

RNA extraction and Northern blot. Total RNA extraction used ULTRASPEC RNA (Biotecx Laboratories, Houston, TX). RNA was also extracted from different organs (brain, heart, liver, lung, kidney, testis, spleen, and thymus) of adult mouse. All RNA samples had an $A_{260/280}$ ratio between 1.8 and 2.0, after the running gel had been checked. Eighteen micrograms of total RNA from different mouse tissues was separated on a 0.8% formaldehyde–agarose gel with ethidium bromide directly added to the loading buffer. The transfer was carried out by capillary transfer overnight. The nitrocellulose membrane was fixed by UV light for 5 min and the prehybridization was done at 68 °C for 1 h in hybridization solution. The filters were then hybridized with the [^{32}P]cDNA probe at 68 °C for 16 h. After washing as recommended by the manufacturer, blots were exposed to X-ray films at −70 °C with an intensifying screen for 1–2 days.

Construction of plasmids and expression of fusion protein. To produce the CK2β protein, the coding region of CK2β cDNA was amplified by polymerase chain reaction (PCR) using primers representing the first and last 20 nucleotides of CK2β. The upstream and downstream primers contained at their 5′ end an *Bam*HI and *Eco*RI restriction enzyme recognition site, respectively. The PCR product was unidirectionally cloned into pGEX2 vector using the restriction sites mentioned and the bacterial strain Top10. The correct sequence of the obtained clone was confirmed. For expression, *Escherichia coli* BL21 was used. The cells were grown at 30 °C to an OD of 0.8, induced with isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM, and further grown at 30 °C for another 5 h, after which the culture was harvested. The recombinant protein was purified on glutathione–Sepharose (Pharmacia) and protein concentrations were determined by the Bradford assay (Bio-Rad).

In vitro binding assays. Tctex4 labeled with [^{35}S]methionine was synthesized by coupled transcription–translation using a TnT Kit (Promega) with T7 polymerase according to manufacturer's instructions. For in vitro binding, 20 μl of the reaction was added to 200 μl of binding buffer (100 mM KCl/10% glycerol/5 mM EDTA/20 mM Hepes, pH 7.6/0.02% NP40/1 mM DTT/5 mg/ml BSA) followed by 10 μl of glutathione–agarose beads with bound GST or GST-CK2β fusion protein and was incubated at 4 °C. The beads were washed three times with binding buffer. Bound proteins were eluted with 2× SDS–PAGE sample buffer and resolved by 12% SDS–PAGE. ^{35}S-labeled bands were detected by autoradiography using a PhosphorImager.

In situ hybridization. In situ hybridization was performed on parafilm sections of adult male mouse testis. Slides were washed in phosphate-buffered saline (PBS; 80 mM Na_2HPO_4, 20 mM NaH_2PO_4, and 100 mM NaCl), fixed in 4% paraformaldehyde, and rinsed in PBS twice. The tissue sections were incubated in the presence of proteinase K for 5 min and then washed in 4% paraformaldehyde (PFA) for 10 min. After a 1-min wash in PBS and deionized H_2O, the sections were dehydrated with four 5-min washes in ethanol (50%, 70%, 90%, and 100%) and air dried. The cDNA fragment of TcTex4 was cloned into the vector pBluescript II KS+ containing two RNA transcription promoters, T3 and T7, to be named pBTctex4. The sense probe was synthesized using T3 RNA polymerase and the plasmid was linearized with *Xho*I, whereas the antisense probe was synthesized using T7 RNA polymerase and the plasmid was linearized with *Eco*RI. ^{35}S-labeled probes (1×10^8 cpm/ml) were dissolved in hybridization buffer [300 mM NaCl, 10 mM Tris–HCl, pH 7.4, 10 mM sodium phosphate, 5 mM EDTA, 100 mM dithiothreitol (DDT), 10% dextran sulfate, 50% formamide, 2 mg/ml bovine serum albumin, 2 mg/ml Ficoll, and 2 mg/ml polyvinylpyrrolidonel], heated at 100 °C for 2 min, applied onto the sections, and covered with siliconized coverslips. Following overnight hybridization at 55 °C, the slices were washed as follows: 5× SSC and

10 mM DDT for 30 min at 37 °C; 50% formamide and 2× SSC for 30 min at 65 °C; 0.5 M NaCl, 10 mM Tris–HCl, pH 7.4, and 4 mM EDTA for 10 min at 37 °C; 0.5 M NaCl, 10 mM Tris–HCl, pH 7.4, 5 mM EDTA, and 0.02 mg/ml RNase A for 10 min at 37 °C; 2× SSC, 10 mM DDT, and 50% formamide for 15 min at 37 °C; 2× SSC for 15 min at 37 °C; and 0.1× SSC for 15 min at 37 °C. For morphological identification, the sections were stained with Giemsa and coverslips were dipped in Eukitt, whereas for autoradiography the slices were dipped in Kodak NTB-2 emulsion diluted 1:1 with water and exposed for 3–5 days.

Results

Isolation and analysis of the cDNA

To isolate the cDNA which encodes for the protein that interacts with CK2β, a yeast two-hybrid system that uses GAL4 recognition site to regulate expression of *his*, *Ade*, and *LacZ* was utilized. The GAL4-DB-CK2β fusion protein did not itself activate transcription of the reporter gene (data not shown). The GAL4-DB-CK2β plasmid pGKT7 was co-transformed with the GAL4-AD-cDNA library of mouse testis pCAT2 into AH 109 yeast strain. Transformants were plated on a minimal medium lacking leucine, tryptophan, adenine, and histine, a conforming Leu–Trp–Ade–His-select medium. From 2×10^6 transformants of murine testis cDNA in pACT2 and CK2β as bait in pGBKT7 clones, 20 positive candidate clones were obtained. Ten clones had increased β-galactosidase activity. Following sequencing these 10 candidates were composed of three CK2α′, two CK2α, and two CK2β, one being A-Raf and two of them identified as a protein which we have designated Tctex4 (t-complex testis expressed 4). CK2α and α′ are known CK2β partners for CK2 holoenzyme; therefore, this result also confirmed the specificity of the interaction, at least for an interacting protein of CK2β. To verify that Tctex4 exists in murine cDNA library, this cDNA was utilized as a probe to screen a mouse testis cDNA library λgt11, and eight clones were selected and sequenced: five were Tctex4 and three were Tctex2 (data not shown). Since Tctex4 has high homology with Tctex2, we conducted BLAST searches of the sequence tagged database

```
1    GCGGCCGCGTCGACCGGAGGCGTCCCGGTGAGCCGATCAAGATGGAGCGGCGAGGCCGAA
                                              M  E  R  R  G  R  M    7
61   TGGCGAAGACGCCCACCGGCCAAACGCATCAATCCCCGGTGTCTAAGAGAGAAAGGAAGC
       A  K  T  P  T  G  Q  T  H  Q  S  P  V  S  K  R  E  R  K  P   27
121  CTAGCATGTTCGAGAAGGAGTCATATGCACAGATCTTAAGAGAAAGACTGAGAGAGTCTT
       S  M  F  E  K  E  S  Y  A  Q  I  L  R  E  R  L  R  E  S  F   47
181  TTCATGATGTTCAGTACGTGGAACCTCCGTTTGATGACTCAATTGCTGATGTAGGCAAAG
       H  D  V  Q  Y  V  E  P  P  F  D  D  S  I  A  D  V  G  K  E   67
241  AATGGAAAAGTGCCCTGGCAAAATTAAAGTTTGCTAATTCATACAGAATGGAGCCACTGA
       W  K  S  A  L  A  K  L  K  F  A  N  S  Y  R  M  E  P  L  K   87
301  AGAAATTTCAAGCACATTTGGTAGAAACTAAAATCCAGCAGATATTAAAGATTGCCAGCA
       K  F  Q  A  H  L  V  E  T  K  I  Q  Q  I  L  K  I  A  S  R  107
361  GATGGATCTGGGATGTGGCATGGGACAACTGGGTAGAAGCTAAACATGAAACAGAGTCTT
       W  I  W  D  V  A  W  D  N  W  V  E  A  K  H  E  T  E  S  Y  127
421  ACGTGGTATTGGCCTTGGTGTTTGCTCTCTATTGTGAATAGCTCANGACCAGCATTTTCC
       V  V  L  A  L  V  F  A  L  Y  C  E  *                      139
481  CCCCCATCCTTCAAAATAAATGATATATACAGATCAAAAAAAAAAAAAAAAAA         540
```

Fig. 1. Nucleotide cDNA sequence of mouse Tctex4. The numbers on the left refer to nucleotide positions and the numbers on the right refer to amino acid positions. The GenBank Accession No. is AY172988.

```
Tctex4      1:  MERRGRMAKTPTGQTHQSPVSKRERKPSMFEKESYAQILRERLRESFHDVQYVEPPFDDS  60
Tcte3       1:  MERRGRMAKTPTGQTHESPVSKSERRPSMFEKESYAQILR-P-RESFRDVQFVEPPFDDA  58
Tctex2      1:  MERRGRMAKTPTGQTHQSPVSKRERKPSMFEKESYAQILRERLRESFHDVQYVEPPFDDS  60
CpG-rich    1:  MERRGQLRKTPTGQTHQSPVSKRERKPSMFEKESYAQILRERLRESFHDVQYVEPPFDDS  60

Tctex4     61:  IADVGKEWKSALAKLKFANSYRMEPLKKFQAHLVETKIQQ---------------------- 100
Tcte3      59:  IADSGKEWKSALAKLKFANSYRMEPLKKFQAHSVETKIQQILKDSLKDVKYDDKAPHLSL 118
Tctex2     61:  IADVGKEWKSALAKLKFANSYRMEPLKKFQAHLVETKIQQILKDSLKDVKYDDKAPHLSL 120
CpG-rich   59:  IADVGKEWKSALAKLKFANSYRMEPLKKFQAHLVETKVQQILKDSLKDVKYDDKAPHLSL 120

Tctex4    101:  ----------------------I--L---K------IASRWIWDVAWDNWVEAKHETESYV 128
Tcte3     119:  ELADRILAAVKEFAYHRYKFIIQVLFIQKTGQGINIASRWIWDVAWDNWVEAKHETESYV 178
Txtex2    121:  ELADGILAAVKEFAYHRYKFIIQVLFIQKTGQAINIASRWIWDVAWDNWVEAKHETESYV 180
CpG-rich  119:  ELADQYWQQSKNFIPWIY-YYTSIIY-SK--DW-S-SNKYCQQMDLG--CGMGQLGRS-- 170

Tctex4    129:  VLALVFALYCE                                                  139
Tcte3     179:  VLALVFALYCE                                                  189
Tctex2    181:  VLALVFALYCE                                                  191
CpG-rich  171:  -----------
```

Fig. 2. Comparison of the amino acid sequence of Tctex4 with those of other homologous proteins. Shown is an optimized multiple sequence alignment created using the GENETYX-MAC 8.0 program. Protein sequences included in the comparison are the translation of the mouse testis cDNA Tctex4 (reported here): Tcte3 (T-complex-associated testis expressed 3, Accession No. NM_011560), Tctex2 (T-complex testis-specific protein, Accession No. U21673), CpG-rich (T-complex, testis-specific protein, Accession No. M26332). Amino acids identical among more than four proteins are shaded to indicate conserved amino acids.

and found that Tctex4 is highly homologous with mouse T-complex testis-specific protein (TcTex2 94% and CpG-rich protein 85%) [10,11] and t-complex-associated testis expressed 3 (Tcte3 93%) [12] as Fig. 2 shows the comparative amino acid sequences.

The open reading frame of clone TcTex4 encoded an 139-amino-acid (as shown in Fig. 1) putative 14-kDa protein from position 42 to 461. The translation initiation site (ATG), assigned to the first methionine codon (nucleotides 41), is in the favorable initiation context, thus fulfilling the criteria for initiation [13].

Expression of Tctex4 in mouse testis

To determine which tissues expressed Tctex4, Northern blotting was performed. Total RNA was isolated from the various organs, separated by agarose gel, blotted onto a nitrocellulose membrane, and probed with ^{32}P-labeled Tctex4 cDNA. Fig. 3 shows that Tctex4 mRNA is expressed by and large only in the mouse testis and not in other tissues. To confirm the expression of Tctex4 mRNA in mouse testis, in situ hybridization was performed. As shown in Fig. 4A, Tctex4 was expressed strongly in the seminiferous tubules of mouse testis. Fig. 4B shows the testis seminiferous tubules stained by H&E (hematoxylin and eosin).

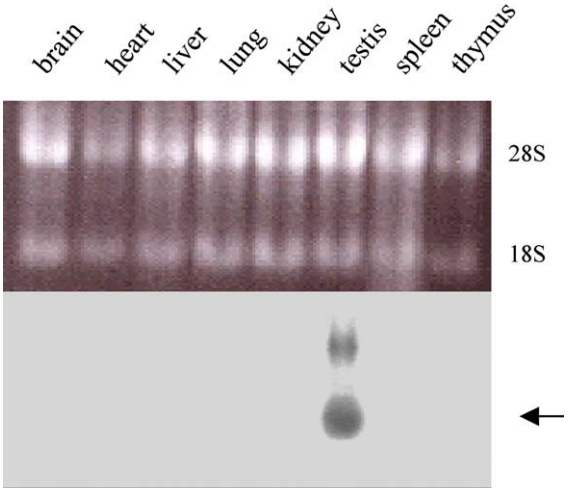

Fig. 3. Expression of Tctex4 mRNA in different mouse organs. The top panel shows total RNA (18 μg) from brain, heart, liver, lung, kidney, testis, spleen, and thymus of mouse loaded in each lane on a 0.8% formaldehyde–agarose gel. The positions of rRNAs are shown on top left. In the bottom is shown Northern blot hybridization with the ^{32}P-labeled Tctex4 probe.

Interaction of CK2β with Tctex4 in yeast

To test the specificity of CK2β binding to Tctex4, we cloned the C- and N-terminals of CK2β into pGKT7 vector as the GAL4-BD-CK2β binding domain. In addition, the full-length cDNA clones of CK2β, CK2α, and CK2α′ were subcloned into the same vector. The GAL4-AD-Tctex4 activation domain in vector pCAT2 and those of various GAL4-BD-CK2β in vector pGKT7 were introduced into yeast strain AH 109. Table 1 shows that the amino-terminus of CK2β interacts with Tctex4 mapping of Tctex4 activity domain in CK2β using the yeast two-hybrid assay. Full-length and deletion constructs of BD-CK2β constructs were screened for interaction with AD-Tctex4 in the yeast two-hybrid assay. Semiquantitative assessment of growth on *His*$^-$/*Leu*$^-$/*Trp*$^-$/*Aden*$^-$ plates was made for 4 days. The intensity of blue color in the LacZ filter assay was determined at the 3-h time point and the β-galactosidase activities are expressed as means of five experiments (±) standard deviation as shown in Table 1. The demonstration has shown that Tctex4 interacts only with CK2β, while the C-terminal is the domain interacting with Tctex4. CK2α and CK2α′ are not able to interact with Tctex4.

Interaction of CK2β with Tctex4 in vitro

To verify the yeast two-hybrid result, a GST-pull down assay was performed. We used Tctex4 in vitro

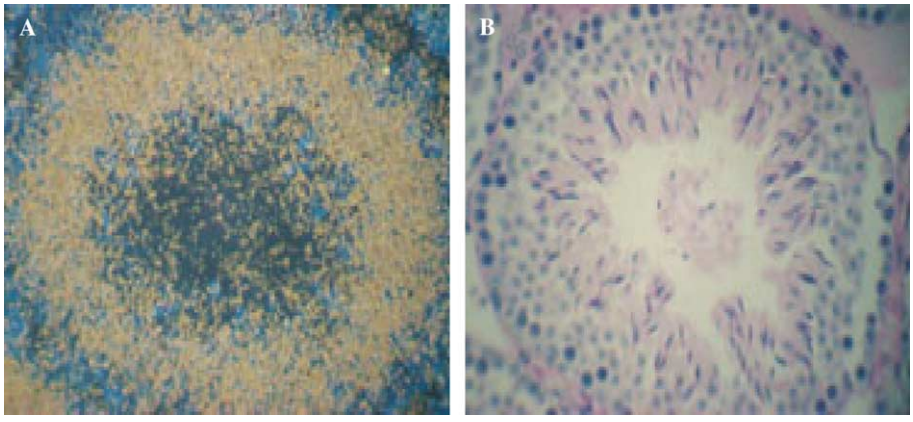

Fig. 4. In situ hybridization analysis of Tctex4 expression in mouse testis section. (A) RNA:RNA hybridization of Tctex4 in mouse testis section; the strong signal shown in a tube of testis tissue. (B) A serial section of testis stained by H&E.

Table 1
CK2 interacts with Tctex4 in yeast two-hybrid assay

Construct	Growth in plates		β-Gal activity	Filter color
	Trp^-/Leu^-	$Trp^-/Leu^-/His^-/Ade^-$		
BD-CK2β	+	+	3.8 ± 0.3	Blue
BD-CK2β108–215	+	+	1.3 ± 0.3	Blue
BD-CK2β1–108	+	–	$\leqslant 0.05$	White
BD-CK2α	+	–	$\leqslant 0.05$	White
BD-CK2α′	+	–	$\leqslant 0.05$	White

The β-galactosidase activities are expressed as means of five experiments (±) standard deviation.

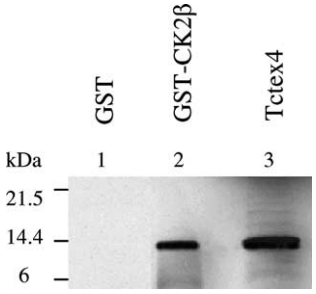

Fig. 5. Autoradiograph of SDS–PAGE analysis showing in vitro interaction of GST-CK2β or GST immobilized on glutathione–agarose beads mixed with ^{35}S-labeled Tctex4 translated. Lane 1, GST protein with Tctex4 translated; lane 2, GST-CK2β fusion protein with Tctx4 translated; and lane 3, Tctex4 translated only. The left shows the Mark12 standard for protein electrophoresis on 12% SDS–PAGE gel.

translated under T7 promoter and [^{35}S]methionine-labeled. After binding to GST-CK2β fusion protein or GST protein and washing 3–4 times, the pellets were dissolved in 1× SDS-sample buffer and separated on 12% SDS–PAGE gel. As shown in Fig. 5, lane 1 is not a signal of the GST protein only; lane 2 shows that the size of band interacting with Tctex4 and translated with GST CK2β is the same as the size of Tctex4 translated alone. This confirms that CK2β interacts with Tctex4 in vitro.

Discussion

In the last few years the number of genes found to be expressed in testis has increased dramatically, especially in the T/t complex, where they have been actively sought [15,16]. This ranges from housekeeping genes to oncogenes to testis-specific genes of known or unknown function. Many can be characterized by stage-specific expression patterns or by unique transcript sizes in testes, and it is generally recognized that almost every transcript made in brain is also expressed in testis. The Tctex2 protein is a sperm tail surface protein mapping to the t-complex in mouse testis and belongs to dynein light chains which associate with the intermediate chain at the base of the soluble dynein particle. These components are essential for dynein assembly and participate in specific motor–cargo interaction [16]. In this study, we have found a novel gene Tctex4 in mouse testis, its protein interacting with CK2β in yeast and in vitro. The C-terminus of CK2β is necessary and sufficient for this interaction. CK2α and CK2α′ could not interact with Tctex4 protein in the yeast two-hybrid assay. BLAST searches of sequence tagged database found that Tctex4 is highly homologous with mouse T-complex testis-specific protein TcTex2 and suggest that CK2 may play an important role in the mechanochemical cycle of cytoplasmic dynein and can bind to cytoplasmic dynein, which should be a significant step toward understanding the regulation of dynein function within the cell.

CK2β is a subunit of CK2, a serine/threonine kinase that can phosphorylate numerous substrates including many signaling proteins and transcription factors [1,17]. The CK2 holoenzyme consists of an $\alpha_2\beta_2$ tetramer of α (or α′) catalytic subunits and β regulatory subunits. The β subunit is critical for stabilizing the structure of the holoenzyme and it contains both an α-binding domain and a β-dimerization domain. β–β dimerization brings two heterodimers (αβ) together to form a tetramer [18]. The regulatory role of the β subunit is complex. Upon binding to CK2β, CK2α acts on most protein and peptide substrates. However, it significantly inhibits calmodulin phosphorylation by CK2α [19].

There are indications that CK2β may have functions other than regulating CK2α activity. CK2β is mainly found in the nucleus partially overlapping with the expression of CK2α [20,21]. However, some nuclear CK2α appears to bind not to CK2β but to the intranuclear components [22] in fission yeast, while overexpression of CK2β causes multiseptation and severe growth defects with no more than twofold increase in CK2 kinase activity. By contrast, overexpression of CK2α has no effect [23].

Our data suggest that the C-terminal region of CK2β is important for Tctex4 binding, which is the same observation that CK2β binds specifically to Mos in yeast [8]. It is possible that the C-terminus of β recognizes the common serine/threonine kinase structures between Tctex4 and CK2α.

In summary, this work has demonstrated that the novel gene Tctex4, its protein interacting with CK2β in a yeast two-hybrid system and in an in vitro interaction assay, is expressed specifically in mouse testis confirmed by in situ hybridization and Northern blot. Our studies

suggest that CK2 may play an import role in the mechanochemical cycle of cytoplasmic dynein and can bind to cytoplasmic dynein, which should be a significant step toward understanding the regulation of dynein function within the cell.

Acknowledgments

We are grateful to Dr. G.A. Carlson, Dr. D. Stephenson, and Dr. B. Provance for many helpful discussions during this study and to Dr. P.X. Xu, Dr. P. Mo, and Dr. W.-M. Zheng for the technical support.

References

[1] L.A. Pinna, Casein kinase II: an eminence grise in cellular regulation, Biochim. Biophy. Acta 1054 (1990) 267–284.
[2] T.L. Yang-Feng, K. Zheng, I. Kopatz, T. Naiman, D. Canaani, Mapping of the human casein kinase II catalytic subunit genes: two loci carrying the homologous sequences for the α subunit, Nucleic Acids Res. 19 (1991) 7125–7129.
[3] B. Boldyreff, C. Klett, E. Gottert, A. Geurts van Kessel, H. Hameister, O.G. Issinger, Assignment of casein kinase 2 α sequences to two different human chromosomes, Hum. Genet. 89 (1992) 79–82.
[4] B. Guerra, O.G. Issinger, Protein kinase CK2 and its role in cellular proliferation, development and pathology, Electrophoresis 20 (1999) 39–408, Review.
[5] M. Orlandini, F. Semplici, R. Ferruzzi, F. Meggio, L.A. Pinna, S. Oliviero, Protein kinase CK2α′ is induced by serum as a delayed early gene and cooperates with Ha-ras in fibroblast transformation, J. Biol. Chem. 273 (1998) 21291–21297.
[6] X. Xu, P.A. Toselli, L.D. Russell, D.C. Seldin, Globozoospermia in mice lacking the casein kinase II α′ catalytic subunit, Nat. Genet. 23 (1999) 118–121.
[7] G. Stalter, S. Siemer, E. Becht, M. Ziegler, K. Remberger, O.G. Issinger, Asymmetric expression of protein kinase CK2 subunits in human kidney tumors, Biochem. Biophys. Res. Commun. 202 (1994) 141–147.
[8] M. Chen, D. Li, E.G. Krebs, J.A. Cooper, The casein kinase II β subunit binds to Mos and inhibits Mos activity, Mol. Cell. Biol. 17 (1997) 1904–1912.
[9] M. Kusk, R. Ahmed, B. Thomsen, C. Bendixen, O.G. Issinger, B. Boldyreff, Interactions of protein kinase CK2β subunit within the holoenzyme and with other proteins, Mol. Cell. Biochem. 191 (1999) 51–58.
[10] L.Y. Huw, A.S. Goldsborough, K. Willison, K. Artzt, Tctex2: a sperm tail surface protein mapping to the t-complex, Dev. Biol. 170 (1995) 183–194.
[11] G.A. Rappold, L. Stubbs, S. Labeit, R.B. Crkvenjakov, H. Lehrach, Identification of a testis-specific gene from the mouse t-complex next to a CpG-rich island, EMBO J. 6 (1987) 1975–1980.
[12] S. Kawakami, K. Mitsunaga, Y.Y. Kikuti, A. Ando, H. Inoko, K. Yamamura, K. Abe, Tctex3, related to *Drosophila* polycomblike, is expressed in male germ cells and mapped to the mouse t-complex, Mamm. Genome 9 (1998) 874–880.
[13] M. Kozak, An analysis of vertebrate mRNA sequences: intimations of translational control, J. Cell Biol. 115 (1991) 887–903, Review.
[14] B. Guerra, S. Siemer, B. Boldyreff, O.G. Issinger, Protein kinase CK2: evidence for a protein kinase CK2β subunit fraction, devoid of the catalytic CK2α subunit, in mouse brain and testicles, FEBS Lett. 462 (1999) 353–357.
[15] Y.I. Yeom, K. Abe, D. Bennett, K. Artzt, Testis-/embryo-expressed genes are clustered in the mouse H-2K region, Proc. Natl. Acad. Sci. USA 89 (1992) 773–777.
[16] L.W. DiBella, S.E. Benashski, H.W. Tedford, A. Harrison, R.S. Patel-King, S.M. King, The Tctex1/Tetex2 classes of dynein light chains, J. Biol. Chem. 276 (2001) 14366–14373.
[17] J.K. Allende, C.C. Allede, Protein kinase CK2: an enzyme with multiple substrates and a puzzling regulation, FASEB J. 9 (1995) 313–323.
[18] R.D. Gietz, K.C. Graham, D.W. Litchfield, Interactions between the subunits of casein kinase II, J. Biol. Chem. 270 (1995) 13017–13021.
[19] A.P. Bidwai, J.C. Reed, C.V. Glover, Phosphorylation of calmodulin by the catalytic subunit of casein kinase II is inhibited by the regulatory subunit, Arch. Biochem. Biophys. 300 (1993) 265–270.
[20] W. Krek, G. Maridor, E.A. Nigg, Casein kinase 2 is a predominantly nuclear enzyme, J. Cell Biol. 116 (1992) 43–55.
[21] P. Lorenz, R. Pepperkok, W. Amsorge, W. Pyerin, Cell biological studies with monoclonal and polyclonal antibodies against human casein kinase II subunit β demonstrate participation of the kinase in mitogenic signaling, J. Biol. Chem. 268 (1993) 2733–2739.
[22] J. Stigare, N. Buddelmeijer, A. Pigon, E. Egyhazi, A majority of casein kinas IIα subunit is tightly bound to intranuclear components but not to the β subunit, Mol. Cell. Biochem. 129 (1993) 77–85.
[23] L. Roussou, G. Draetta, The *Schizosaccharomyces pombe* casein kinase II α and β subunits: evolutionary conservation and positive role of the β subunit, Mol. Cell. Biol. 14 (1994) 576–586.