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RESEARCH ARTICLE

Protein kinase CK2 and new binding partners during spermatogenesis

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Abstract Protein kinase CK2 is an ubiquitously expressed enzyme that is absolutely necessary for the survival of cells. Besides the holoenzyme consisting of the regulatory β-subunit and the catalytic α- or α'-subunit, the subunits exist in separate forms. The subunits bind to a number of other cellular proteins. We show the expression of individual subunits as well as interaction with the transitional nuclear protein TNP1 and with the motor neuron protein KIF5C during spermatogenesis. TNP1 is a newly identified binding partner of the α-subunit of CK2. CK2α and KIF5C were found in late spermatogenesis, whereas CK2β and TNP1 were found in early spermatogenesis. CK2α, CK2α', TNP1, and KIF5C were detected in the acrosome of spermatozoa, while CK2β was detectable in the mid-piece. Combinations of CK2 subunits might determine interactions with other proteins during spermatogenesis. KIF5C as a kinesin motor neuron protein is probably involved in the redistribution of proteins during spermatogenesis.

Keywords Protein kinase · Transmission electron microscopy · Immune histochemistry · TNP1 · KIF5C · Spermatogenesis

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Introduction

Protein kinase CK2 (formerly known as casein kinase 2) is a serine/threonine kinase found ubiquitously in eukaryotes from yeast to man. CK2 can use both ATP and GTP as phosphate donors. It preferentially phosphorylates serine/threonine residues in the vicinity of acidic amino acids. The holoenzyme is a heterotetramer consisting of two catalytic α- or α'-subunits and two regulatory β-subunits [1, 2]. There is increasing evidence that the individual subunits possess functions that are different from their functions in the holoenzyme. CK2 phosphorylates more than 300 proteins that are implicated in cellular processes as diverse as replication, transcription, translation, signal transduction, and programmed cell death [3]. CK2 regulates the activity of transcription factors, growth factor receptors, and cytoskeletal proteins. Furthermore, it is associated with responses to DNA damage or with the regulation of apoptosis [4, 5].

Interestingly, the individual subunits are localized within different cellular compartments, supporting the idea that the CK2 subunits have separable functions [6]. Some substrates are phosphorylated by CK2α or CK2α', but not by the holoenzyme and vice versa [7, 8]. The regulation of these distinct pools of CK2 activity is currently not well understood. Further support for the idea of individual subunit functions comes from CK2α knock-out mice, which are not viable. The absence of CK2α or CK2β leads to early embryonic lethality [9, 10]. However, CK2α' knock-out mice are viable but male mice are infertile [11]. CK2α' is highly expressed in both mice testis and brain [12].

A rapidly increasing number of proteins are known to interact with the individual subunits. In a yeast two-hybrid screen we identified a member of the nuclear

transition proteins TNP1 as a new binding partner for the α-subunit of CK2. In mammals the transition nuclear proteins (TNPs) appear to be intermediate proteins in the histone to protamine transition during spermatogenesis [13]. TNPs are highly expressed at mid-spermatogenesis steps, coincidental with chromatin remodeling. In addition, microarray data show that not only CK2 but also TNP1 is highly expressed in pachytene spermatocytes and round spermatids [14, 15]. Here, we first examined interactions of CK2α, CK2α′, and CK2β subunits with TNP1.

KIF5C is a member of the kinesin-1 heavy chain family, which are motor proteins that transport specific cargoes along the microtubules. The KIF5C gene codes for a protein with 956 amino acids and a molecular weight of 107 kDa. The kinesin heavy chain protein KIF5C is expressed in brain, spinal cord [16], the retina [17], and also in spermatids [18]. We recently found that the KIF5C protein, at least in neurons, preferentially bound to CK2α′ [19]. Here, we applied immunohistochemistry and immunogold labeling to examine the distribution of CK2 subunits and their interacting partners TNP1 and KIF5C in mouse testis. The results provide new insights into the roles of these components during spermatogenesis.

We found supporting evidence that KIF5C also binds to CK2α and that it is co-localized with CK2α at the acrosomes of spermatids. We found a similar distribution of CK2α and KIF5C in late spermatogenesis, whereas CK2β was found in early steps of sperm development. TNP1 was detectable in spermatocytes, but not in elongated spermatids.

Materials and methods

Antibodies

For the detection of CK2 by Western blot, immunofluorescence, and immunoelectron microscopy, we used rabbit serum #26 for CK2α, serum #30 for CK2α′, and serum #32 for CK2β. These sera were raised against amino acids 360–371 of CK2α, amino acids 330–349 of CK2α′, and amino acids 206–215 of CK2β, and have already been described [20]. In addition to Western Blot these antibodies were also used in immune histochemical analysis and immunofluorescence [21, 22]. KIF5C was detected with the rabbit antibody #976, which was raised against a KIF5C-specific peptide with the amino acid sequence CDNTPIIDNIAPV [19]. A highly purified TNP1 protein was used to immunize a rabbit. Rabbit serum #770 was used for all experiments described in the present study.

Plasmids, protein expression, and purification

The plasmid pCEP4 containing the cDNA for murine TNP1 was kindly provided by D. Levesque, Sherbrooke University, Canada [23]. The cDNA was cloned into pGEX4T-1 to yield pGEX4T-1-TNP1. The protein was expressed in *Escherichia coli* BL21 (DE3) cells and purified on GSH Sepharose (GE Healthcare) according to the instructions of the manufacturer. In order to construct GST-CK2α′, we introduced Bam H1 and Not1 restriction sites at the 5′- and 3′- ends of the CK2α′ cDNA, respectively, in a PCR reaction, using an eukaryotic CK2α′ expression vector as template. We cloned the full-length cDNA of CK2α′ (1,050 bp) in frame with the GST cDNA of the pGEX-4T-1 vector from GE Healthcare. The construct was checked by DNA sequencing.

Pull-down assays

GST-TNP1 or GST-tag was bound to GSH Sepharose and washed with PBS to remove all non-bound proteins. Equal molar amounts of CK2α, CK2β, or CK2 holoenzyme, diluted in PBS and 0.6% CHAPS (Roth, Germany), were incubated with TNP1 or the GST-tag for 2 h at 4°C. The Sepharose was washed with NET buffer (150 mM NaCl, 5 mM Na-EDTA, 50 mM Tris–HCl, pH 7.4, 5% sucrose, 1% NP40) and finally with PBS. Bound proteins were eluted with 30 µl of SDS sample buffer (130 mM Tris–HCl, pH 6.8, 0.02% Bromophenol Blue (w/v), 10% 2-mercaptoethanol, 20% glycerol, 4% SDS) at 95°C for 10 min. Alternatively, rabbit anti-CK2α′ antibody was bound to protein A Sepharose at 4°C for 1 h. Unbound antibody was removed by washing three times with PBS. In the next step, GST-CK2α′ was bound to the antibody. After extensive washing with PBS, GST-CK2α′was incubated with GST-TNP1 or, as a control, with GST-CK2β or with the GST-tag alone. Complexes were washed and eluted as described above.

Kinase assay

Purified cyclin H (0.5 µg) diluted in kinase buffer (50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT) were incubated with 1 mM ATP and 2 µCi $^{32}P\gamma ATP$.

GST-TNP1 and the GST-tag as a control were added in increasing concentrations upon commencement of the kinase reaction. After 30 min at 37°C, the kinase reaction was stopped by adding sample buffer. Proteins were analyzed on a 12.5% SDS polyacrylamide gel.

Sperm and tissue preparation, immunohistochemistry

Cauda epididymal sperm from male mice (Swiss Webster, retired breeders) were prepared as described in previous

works [24, 25]. Animals were euthanized by CO_2 asphyxiation and epididymides were excised. After removal of adipose tissue, epididymides were cleaned with Na7.4 medium (135 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM HEPES, 5 mM glucose, 10 mM lactic acid, 1 mM pyruvic acid, adjusted to pH 7.4 with NaOH). After 5 to 10 small incisions, tissue was transferred to HSB (Na7.4 medium with 15 mM $NaHCO_3$ and 5 mg/ml BSA). Spermatozoa were allowed to exude for 15 min (37°C, 5% CO_2). Sperm were washed twice with Na7.4. For tissue preparation, testes were removed and fixed in Bouin solution (6–8 h at room temperature) and in 70% isopropanol (12 h at room temperature). After dehydration in ascending isopropanol series and xylol, tissue samples were embedded in paraffin and sectioned into 5-µm slices, which were then mounted on silanized slides. For DAB immunostaining, dewaxed and rehydrated samples were incubated at 4°C overnight with the primary antibodies (CK2α 1:200, CK2α' 1:50, CK2β 1:50, TNP1 1:200, and KIF5C 1:200). Washing in PBS was followed by incubation of the slides for 30 min at room temperature with biotinylated secondary antibodies diluted 1:200 in PBS/biotin (1:50)/NGS (5%). After washing in PBS, the Vectastain® ABC elite kit for peroxidase was applied and peroxidase activity was determined using diaminobenzidine (DAB) as chromogen. Nuclei were stained with hematoxylin and slides were subsequently dehydrated, embedded in Depex, and examined under a Zeiss Axiophot microscope equipped with a Color View camera (Soft Imaging Systems, Münster, Germany). Negative controls were incubated with an unspecific primary antibody. For immunofluorescence studies, sperm smears were prepared by using 20 µl of sperm suspensions. After air-drying, the smears were fixed in acetone (4°C, 1 min). All subsequent steps were performed in a dark moist chamber at room temperature. Incubation steps were as follows: Blocking with swine serum in PBS (1:500, 1 h), primary antibody (overnight, 4°C; dilutions see DAB immunostaining), Cy3-labeled anti-rabbit IgG (1:50, 1 h), DAPI (1:1,000, 10 min). Each incubation step was followed by three washes with PBS for 5 min each time. Control staining was carried out by replacing the specific primary antibody by a non-relevant primary antiserum or by PBS. Slides were covered in 80% glycerol in PBS and photographed with a Nikon TE-2000U Eclipse invertoscope equipped with a Nikon D1 camera.

Transmission electron microscopy

For immunogold labeling, spermatozoa were used directly after swim-out. Samples were incubated with the primary antibodies, diluted in PBS (overnight, 4°C; dilutions see immunohistochemistry). In control incubations, the primary antiserum was replaced by an unspecific antibody. Sperm samples were washed three times with PBS by centrifugation (300 × *g*, 5 min) and incubated for 1 h at 37°C with anti-rabbit IgG, labeled with 5-nm gold particles in a 1:20 dilution in PBS. After three subsequent washing steps, a part of the sperm samples was incubated for 30 min in a 1% OsO_4 solution in sodium cacodylate buffer (0.5 M, pH 7.3) and washed twice with sodium cacodylate buffer (0.1 M, pH 7.4). The residual pellet was left untreated as a control. Both samples were then dehydrated in a graded series of ethanol, propylene oxide, permeated with an epon-propylene oxide mixture (6 h) and then embedded in fresh epon. Epon was allowed to polymerize at 60°C for 3 days. Ultrathin sections were cut at 500–700 nm on a Reichert ultra microtome equipped with a diamond knife. Sections were collected on uncoated 100-mesh copper grids and briefly stained with uranyl acetate and lead citrate. To examine protein distribution in the testis, we followed a post-embedding protocol, i.e., tissue was first embedded and sectioned, whereas immunostaining was performed afterwards directly on the grids. Meshes were examined with a Tecnai 12BT electron microscope (FEI, Kassel, Germany).

Purification of CK2 subunits and holoenzyme

Recombinant CK2 holoenzyme cloned in a bicistronic vector [26] and the three different subunits (α, α', and β in pT7-7) of protein kinase CK2 were expressed in *Escherichia coli* BL21 (DE3) and the proteins were purified according to published protocols [27, 28].

SDS polyacrylamide gel electrophoresis and Western blot

Proteins were analyzed in 12.5% SDS polyacrylamide gels [29]. For Western blot analysis 50–100 µg of protein extract were transferred to a PVDF membrane by tank blotting with 20 mM Tris–HCl, pH 8.8, and 150 mM glycine as transfer buffer. The membranes were blocked in PBS with 0.1% Tween-20 and 5% dry milk for 1 h at room temperature. The membrane was incubated with the primary antibodies diluted in PBS-Tween-20 with 1% dry milk, either overnight at 4°C or for 1 h at room temperature. Serum #26 directed against CK2α and serum #32 directed against CK2β were used in a dilution of 1:1,000 overnight. TNP1 was detected with a GST-specific rabbit serum in a dilution of 1:1,000. Rabbit antibody #976 [19] was used for the detection of KIF5C. After washing with PBS-Tween-20 with 1% dry milk, the membrane was incubated with the peroxidase-coupled secondary antibody in a dilution of 1:10,000 (anti-mouse) or 1:30,000 (anti-rabbit) for 1 h at room temperature. The membrane was

washed in PBS-Tween-20 and signals were developed and visualized by the Roche Diagnostics Lumilight system (Mannheim, Germany).

Results

Protein kinase CK2 is involved in various regulatory pathways in the cell. Some of these functions are obviously regulated by the interaction of individual subunits of CK2 with other cellular proteins [30]. In order to detect new CK2-binding partners, we performed a yeast two-hybrid screen with CK2α as bait. In the course of these studies we identified for the first time the transitional nuclear protein 1 (TNP1) as a binding partner for CK2α. In order to verify this interaction we performed pull-down assays with GST-tagged TNP1 and the individual subunits of CK2, namely α, β, and the holoenzyme. Proteins were expressed in bacteria and purified according to conventional procedures. Purified proteins were analyzed on a 12.5% SDS polyacrylamide gel and visualized with Coomassie Blue staining of the polyacrylamide gel (Fig. 1a). Equal amounts of the CK2 subunits were incubated with equal amounts of GST-TNP1 or GST-tag as a control. As shown in Fig. 1b, TNP1 bound to CK2α (lane 1) and to the holoenzyme (lane 1), whereas there was no binding to CK2β (lane 1). As a further control, we showed that there was no binding to the GST-tag alone (lane 2). CK2α′ is mostly insoluble and hard to purify [31], therefore binding to CK2α′ was analyzed with GST-tagged CK2α′ bound to protein A Sepharose-bound antibody #30 against CK2α′ and GST-tagged TNP1(lane 1) or GST-tag (2). As a control, protein A Sepharose-bound CK2α′-specific antibody #30 was incubated with the GST-tag (3). Bound proteins were analyzed on a 12.5% SDS polyacrylamide gel and visualized by an antibody against GST-tag. Figure 1c shows only CK2α′ but no bound GST-TNP1 protein. To ensure that the GST-tag does not interfere with CK2α′, we performed a pull-down experiment with GST-CK2β and GST-CK2α′ under identical conditions as described above. As shown in Fig. 1d, GST-CK2β bound to GST-α′ (lane 3) but not to the CK2α′ antibody alone (lane 4). As a further control, we also showed that GST-CK2α′ did not bind to the GST-tag alone (lane 1) and the GST-tag did not bind to the CK2α′ antibody alone (lane 2). Thus, from these experiments we conclude that TNP1 binds to CK2α but does not bind to either the β-subunit or to CK2α′.

Since CK2α represents the catalytic subunit of CK2, we reasoned that TNP1 might influence the enzyme activity of CK2α. For this type of analysis, we performed an in vitro kinase reaction with the CK2 holoenzyme and increasing concentrations of GST-TNP1, with cyclin H as a substrate. As a control, we also used the GST-tag alone with CK2 and

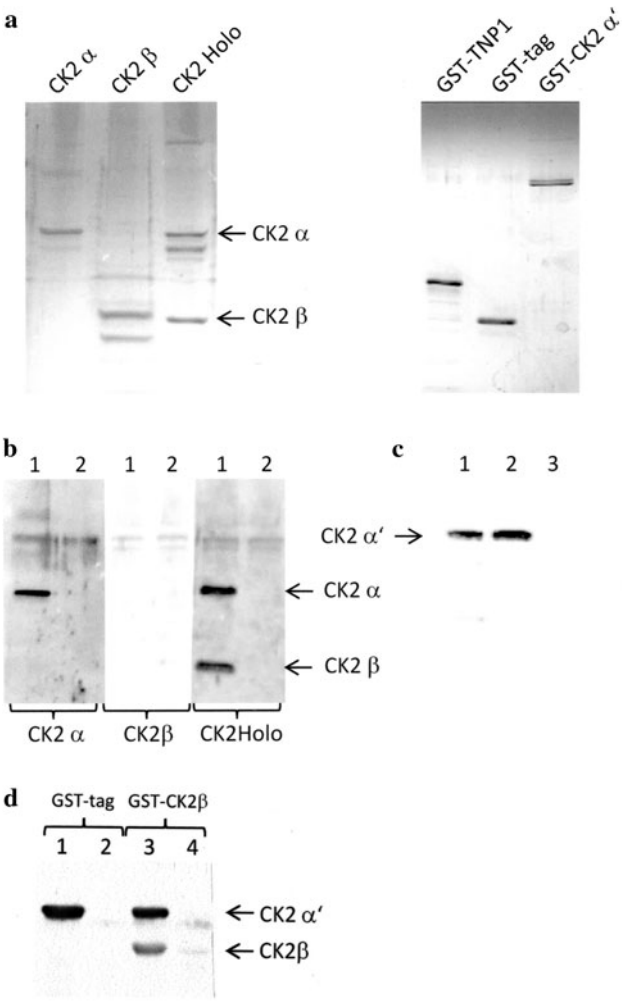

Fig. 1 TNP1 binds to CK2α and the holoenzyme but not to CK2β and CK2α′. **a** Coomassie Blue-stained gel of the purified proteins used for the binding assay. **b** CK2α, CK2β, and CK2 holoenzyme consisting of $CK2\alpha_2\beta_2$ were incubated with GST-tagged TNP1. After extensive washing, protein complexes were collected at GSH Sepharose. Bound proteins were analyzed on a 12.5% SDS polyacrylamide gel. CK2 subunits were identified with antibody #26 against CK2α and #32 against CK2β. **c** Protein A Sepharose-bound CK2α′-specific antibody #30 was incubated with GST-CK2α′ and then with GST-TNP1 (*1*) or GST-tag (*2*). As a control, protein A Sepharose-bound CK2α′-specific antibody #30 was incubated with GST-tag (*3*). Proteins were analyzed on a 12.5% SDS polyacrylamide gel and finally visualized with an antibody against the GST-tag. **d** Protein A Sepharose bound CK2α-specific antibody #30 was incubated with (*1*, *3*) or without GST-CK2α′(*2*, *4*) and then with GST-CK2β (*3*, *4*) or with the GST-tag alone (*1*, *2*). Proteins were analyzed on a 12.5% SDS polyacrylamide gel and finally visualized with an antibody against the GST-tag

cyclin H. As shown in Fig. 2, we found no differences in the phosphorylation of cyclin H, neither in the presence of increasing concentrations of GST-TNP1 nor in the presence of the GST-tag. The same results were observed for the autophosphorylation of the β-subunit. We also

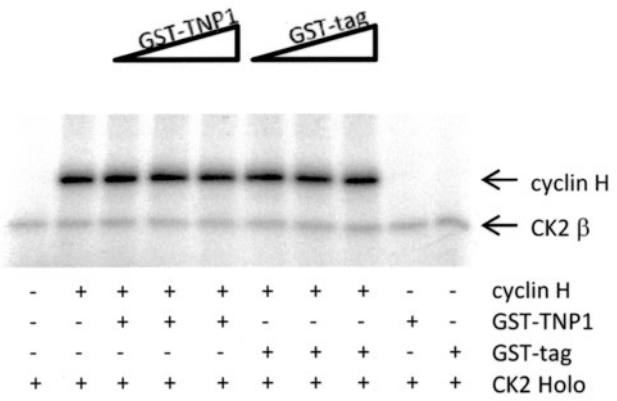

Fig. 2 Influence of increasing concentrations of TNP1 on CK2 kinase activity. Purified CK2 holoenzyme was incubated with $^{32}P\gamma ATP$ with cyclin H as a substrate in the absence or presence of increasing concentrations of TNP1. As a control, we used the GST-tag or GST-TNP1. Phosphorylated proteins were analyzed on a 12.5% SDS polyacrylamide gel, followed by autoradiography

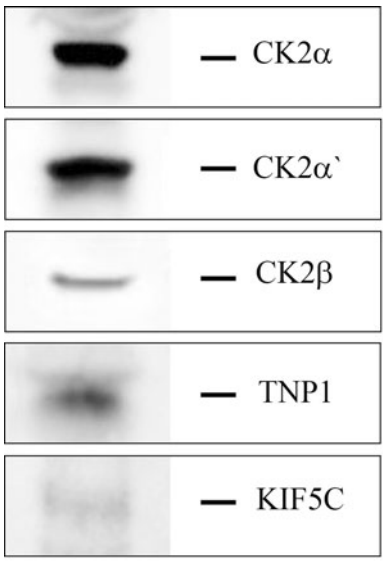

Fig. 3 Western blot analysis of CK2 subunits, TNP1, and KIF5C in spermatozoa. Spermatozoa extract (100 µg) was subjected to a 12.5% SDS polyacrylamide gel. After transfer of the proteins to a PVDF membrane, CK2α, CK2α', CK2β, TNP1, and KIF5C were detected with the corresponding specific antibodies

deliberated whether TNP1 might be a substrate for CK2. Therefore, TNP1 was incubated with CK2 and $^{32}P\gamma ATP$. Proteins were analyzed on a 12.5% SDS polyacrylamide gel. Figure 2 (two lanes to the right) shows that GST-TNP1 was not a substrate for CK2.

Next, we decided to investigate whether all CK2 subunits and TNP1 are expressed in epididymal spermatozoa. Since we had already identified KIF5C as a new binding partner preferentially for CK2α' at least with regard to neurons, we also analyzed KIF5C expression in 100 µg of total protein extract from mouse epididymal spermatozoa. After SDS polyacrylamide gel electrophoresis and transfer of the proteins to a PVDF membrane, proteins were detected with specific antibodies. We found a strong expression of CK2α, α' and TNP1, a considerably lower expression for CK2β, and a very weak expression for KIF5C (Fig. 3).

Having shown that all five proteins are present in spermatozoa, we then analyzed the expression of these proteins in mouse testis. With DAB immunostaining we found the same distribution for CK2α and KIF5C during late spermatogenesis. The immunoreactions visible by brown staining appeared in the acrosome of spermatids (Fig. 4). Immunoreaction with CK2α' also appeared in late spermatogenesis but is not strictly localized at the acrosomal region. CK2β was found in early spermatogenesis, in spermatogonia, and in spermatozytes. No immunoreactions were found for CK2β in the late stages of spermatogenesis. TNP1 was also found in early spermatogenesis and in spermatogenic cells but was no longer displayed in spermatids.

Next, we performed an immunofluorescence analysis for CK2 subunits, TNP1, and KIF5C in spermatozoa. CK2α-, CK2α'-, TNP1-, and KIF5C-antibodies labeled the acrosome of murine spermatozoa (red) (Fig. 5). CK2α immunoreactions were also detected in the mid-piece of the tail. CK2β-specific antibodies led to a weak staining of the acrosome and a strong immunoreaction at the mid-piece. Nuclei were stained with DAPI (blue).

Finally, we proceeded to identify the subcellular distribution of the five different proteins by transmission electron microscopy of spermatids and spermatozoa. Immunoreactions with the CK2α-specific antibody were visible as gold particles (5 nm) in the developing acrosome of spermatids (a–c; c = magnification of b) and in the nucleus of spermatozoa (d) (Fig. 6). A weak immune reaction was found with the CK2β-specific antibody in the main piece of spermatozoa but not in the acrosome (not shown). KIF5C antibodies labeled the developing acrosome of spermatids and spermatozoa (e–g) and mitochondria of the mid-piece (H).

Discussion

Protein kinase CK2 is an ubiquitously expressed enzyme from yeast to man. In mammalian cells, the most abundant form of protein kinase CK2 is a holoenzyme consisting of two regulatory β-subunits and two catalytic α- or α'-subunits. However, there is an increasing number of publications showing that the CK2 subunits are differently expressed and localized in different cell types. One reason

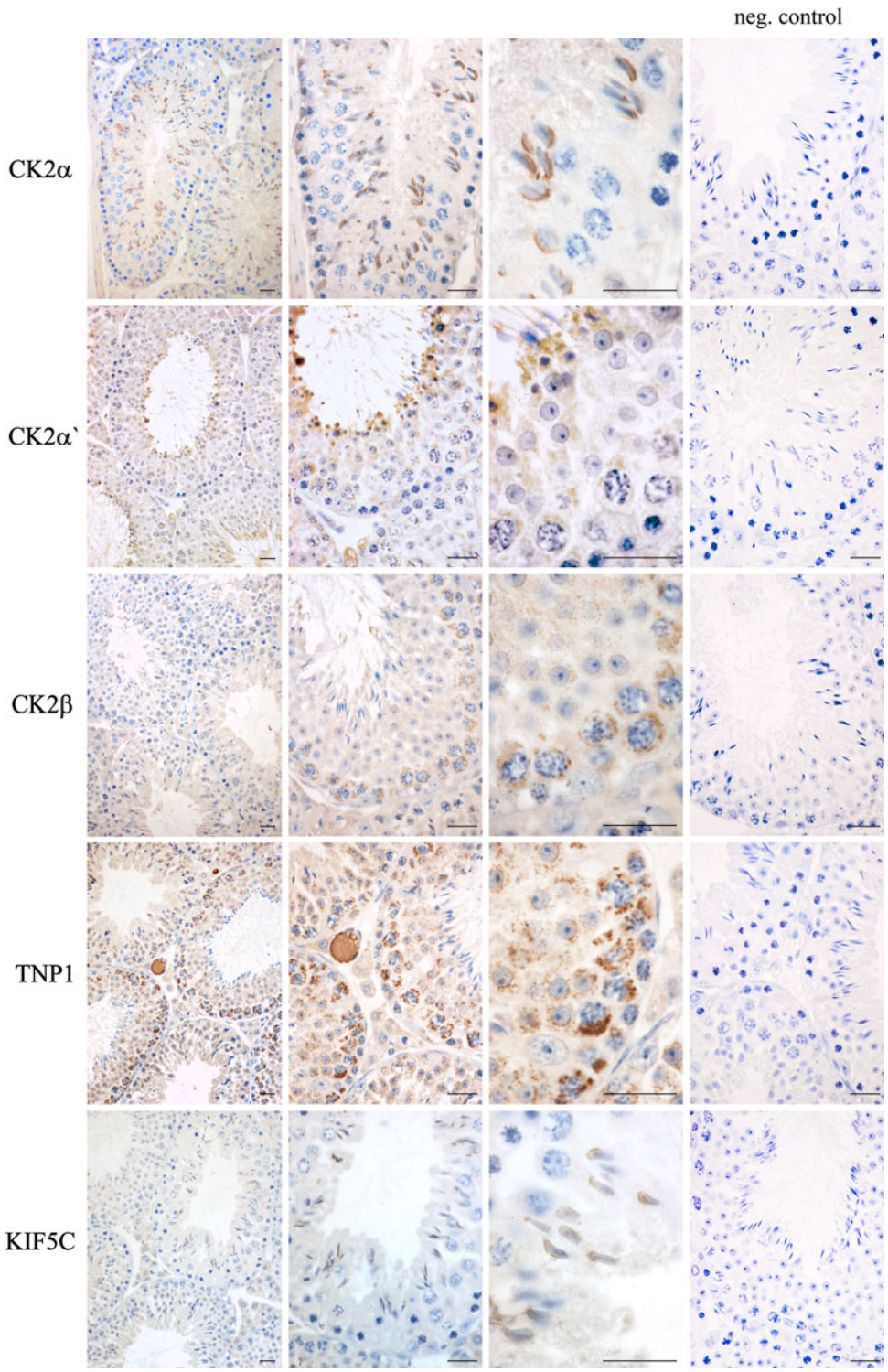

Fig. 4 Immunolocalization of CK2 subunits, TNP1, and KIF5C in mouse testis. CK2α, CK2α', CK2β, TNP1, and KIF5C were localized in mouse testis with DAB immunostaining. CK2α and KIF5C showed the same distribution during late spermatogenesis. The immunoreactions (visible in *brown* staining) appeared in the acrosome of spermatids. CK2α' is also localized in late spermatogenesis. In addition to the acrosomal region, the staining is also visible in the cytoplasm. In contrast, CK2β was localized in early spermatogenesis in spermatogonia and spermatozytes. No immunoreactions were found with CK2β in later stages of spermatogenesis. TNP1 was also found in early spermatogenesis and in spermatogenetic cells, but was no longer displayed in elongated spermatids. Corresponding control stainings with an unspecific antibody are shown on the last picture of each row (*bars* 20 μm)

for this expression pattern might be due to the interaction of the individual subunits with other cellular proteins. Recently, we detected KIF5C as a new binding partner for the catalytic subunits of CK2 [19]. Here, we show that KIF5C was also expressed in spermatozoa. Immunostaining experiments showed the same distribution for CK2α and KIF5C during late spermatogenesis and in particular in the acrosome of spermatids, indicating a specific function of the two proteins not only in neurons. Furthermore, we detected another protein, namely TNP1, in complex with CK2α. The transition nuclear proteins constitute 90% of the chromatin basic proteins during the steps of spermatogenesis between histone removal and deposition of protamines. Only two major TNPs, TNP1 and TNP2, are prominent in

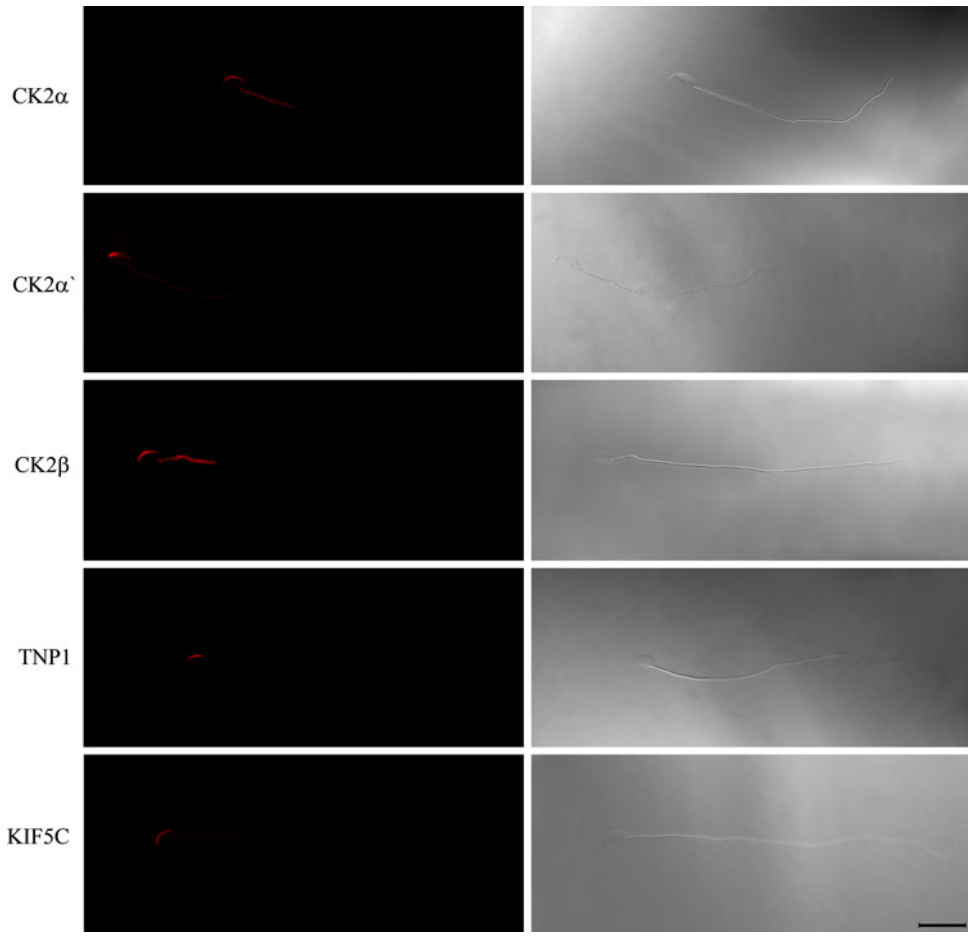

Fig. 5 Immunofluorescence analysis of CK2 subunits, TNP1, and KIF5C in spermatozoa. Epididymal spermatozoa were used for immunolocalization of CK2, TNP1, and KIF5C. CK2α, CK2α', TNP1, and KIF5C antibodies labeled the acrosome of murine spermatozoa (Cy3/*red*). In addition, CK2α and CK2β immunoreactions were also detected in the mid-piece of the tail. On the *right* side of the *panel*, corresponding DIC images are shown. Control staining with an unspecific primary antibody did not show any immunoreactions (not shown; *bar* 20 μm)

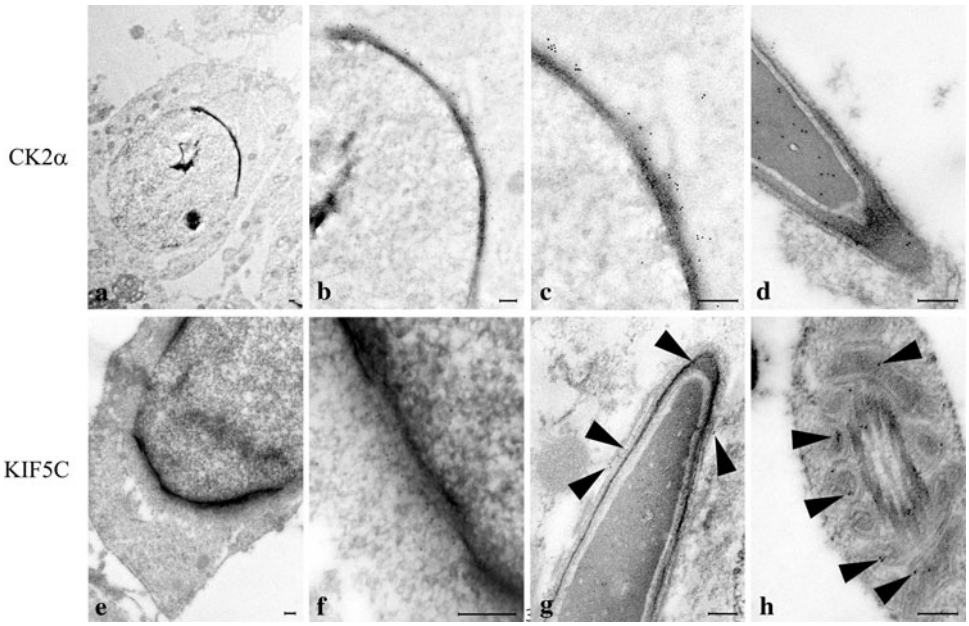

Fig. 6 Transition electron microscopy of spermatids and spermatozoa for the detection of CK2 and KIF5C. Antibodies against CK2, TNP1, and KIF5C were used to identify the subcellular distribution of their corresponding proteins in spermatogenesis. Immunoreactions with the CK2α antibody are visible as *gold* particles (5 nm) in the developing acrosome of spermatids (**a**–**c**; **c** = magnification of **b**) and in the head of spermatozoa (**d**). KIF5C antibody labeled the developing acrosome of spermatids and spermatozoa (**e**–**g**) and mitochondria of the mid-piece (**h**). No, or only weak, immunoreactions were found with CK2α', CK2β, and TNP1 antibodies with electron microscopy (not shown; *bar* 200 nm)

rodent spermatids [32]. TNP1 is a 6,200-Da protein with approximately 20% arginines and 20% lysines, distributed uniformly over the polypeptide chain [33]. TNP1 is abundantly expressed and its sequence is highly conserved in various mammals [34]. Since TNP1 appears mainly in the middle of spermatid maturation, CK2α interaction might play a role at this stage of maturation. As shown here, TNP1 is not a substrate for CK2, which excludes CK2 regulation of TNP1 functions by protein phosphorylation. Instead, it might well be that TNP1 is involved in targeting CK2α to a specific substrate during maturation of spermatids. Interestingly, TNP1-deficient mice show abnormal spermatogenesis, sperm mobility is drastically reduced, and 60% of TNP1 null males were infertile [35]. A similar observation was made for CK2α' knock-out mice, i.e., male mice in which CK2α' has been disrupted, are infertile [11]. The number of epididymal spermatozoa is less than 30% that of controls, sperm cells exhibit head-shape abnormalities and many acrosomes are separated from the nucleus [12], similar to those described in human globozoospermia. Several motor proteins for the redistribution of proteins during spermatogenesis have been established, such as kinesin-II and cytoplasmic dynein 1b [36]. Especially KIF5C could play a major role in the nucleocytoplasmic exchange activities during spermatogenesis.

There are several reports showing that naturally occurring polyamines such as spermine and other proteins rich in lysines, such as histones and polylysine, can activate CK2 kinase activity [37–39]. The activation is mediated through the interaction of the polyamines with the acidic cluster of CK2β. CK2α in the absence of CK2β or in the presence of CK2β that had been mutated in those acidic residues, is no longer stimulated by these polycations [39]. However, as shown here, TNP1 has no influence on CK2 enzyme activity, at least not with regard to the phosphorylation of cyclin H. The present data further support the theory about individual roles of the CK2 subunits at least in spermatogenesis.

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