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Research Article

KIF5C: A new binding partner for protein kinase CK2 with a preference for the CK2α' subunit

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Abstract. Protein kinase CK2 is a highly conserved serine/threonine kinase that is ubiquitously expressed in eukaryotic cells. CK2 is a constitutively active tetrameric enzyme composed of two catalytic α and/or α'-subunits and two regulatory β-subunits. There is increasing evidence that the individual subunits may have independent functions and that they are asymmetrically distributed inside the cell. To gain a better understanding of the functions of the individual subunits, we employed a yeast-two-hybrid screen with CK2α and CK2α'. We identified the motor neuron protein KIF5C as a new binding partner for CK2. The interaction found in the yeast-two-hybrid screen was confirmed by co-sedimentation analysis on a sucrose density gradient and by co-immunoprecipitation analysis. Pull-down experiments and surface plasmon resonance spectrometry revealed a direct binding of KIF5C to CK2α'. Co-localization studies with neuroblastoma cells, bone marrow and with primary neurons confirmed the biochemical analysis that KIF5C preferentially bound to CK2α'.

Keywords. Protein kinase CK2, kinesin, protein-protein interaction, motor neuron protein, CK2 isoforms.

Introduction

Reversible phosphorylation of proteins is the major mechanism for the regulation of a broad spectrum of fundamental cellular processes. Therefore, protein kinases have attracted much attention over the last five decades. One protein kinase known for more than 50 years is protein kinase CK2 (CK2; formerly known as casein kinase II) [1]. CK2 is distributed ubiquitously in eukaryotic organisms where it mostly appears as a heterotetramer consisting of two catalytic α or α'-subunits and two regulatory β-subunits. An additional third catalytic isoform α'' has recently been described [2]. In *Saccharomyces cerevisiae* disruption of the genes coding for the catalytic subunits is lethal. It was further shown that disruption of the regulatory β-subunit [3] as well as disruption of the α-subunit of CK2 is lethal during mouse embryogenesis [4]. These results reinforce the importance of CK2 for the maintenance of cell viability in normal cell life and in embryogenesis [3, 5]. The list of potential physiological substrates is continuously growing [6]. From the various substrates of CK2, it is evident that CK2 regulates a diverse selection of cellular processes [7]. Although CK2 is implicated in so many cellular

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processes, little is known about its own regulation. There are at least three well known functions of the regulatory β-subunits, such as (i) modulation of the activity of the CK2α- or α'-subunits in a substrate-dependent manner [8, 9], (ii) contribution to the substrate specificity [9], and (iii) increasing the stability of the α-subunit [10]. In addition, it appears that a number of distinct mechanisms contribute to the regulation of CK2 including regulated expression and assembly, regulation by covalent modification and regulatory interactions with proteins. So far none of the binding partners of CK2α seems to be able to regulate CK2 activity in a way similar to the regulatory β-subunit. However, there is ample evidence that some binding partners of CK2 might regulate cellular functions of CK2α by targeting or anchoring CK2α to specific cellular locations. Another class of proteins binding to CK2α seems to function as adapters to integrate CK2 in cellular signal transduction pathways [11]. Heat shock proteins such as HSP90 bind to CK2α, and thus prevent self-aggregation of CK2α [12, 13]. Finally, the association of CK2 with cellular proteins might serve bringing the enzyme and the substrate into close contact.

To find new binding partners for the catalytic subunits of CK2, we started a yeast-two-hybrid screen with CK2α as bait. We found over 100 different genes whose products bound to the CK2α subunit in a very stringent re-screen. One of the clones that repeatedly bound to CK2α belongs to the gene coding for KIF5C. KIF5C is a member of the kinesin-1 heavy chain family, which are motor proteins that transport specific cargoes along the microtubules. In addition to KIF5C, the kinesin-1 family members include KIF5A and KIF5B. KIF5B is ubiquitously expressed, whereas KIF5A and KIF5C are only present in neurons [14]. Kinesin-1 proteins are tetramers formed by the association of two kinesin heavy chains and two kinesin light chains. The kinesin heavy chain is composed of an N-terminal motor domain that contains the microtubule- and ATP-binding sites. The C-terminal non-motor domain includes a neck, a coiled-coil stalk region and a cargo-binding site.

KIF5C moves towards the plus end of microtubules and participates in the anterograde transport, selectively transporting molecules from the cell body to axons and dendrites. The KIF5C gene codes for a protein with 956 amino acids and a molecular mass of 107 kDa. The KIF5C protein is expressed in brain, spinal cord [15] and the retina [16]. In the spinal cord, KIF5C is mainly found in motor neurons. Thus, an interaction of CK2 with KIF5C might indicate a novel function for CK2 in the nervous system, and therefore this interaction was investigated in more detail.

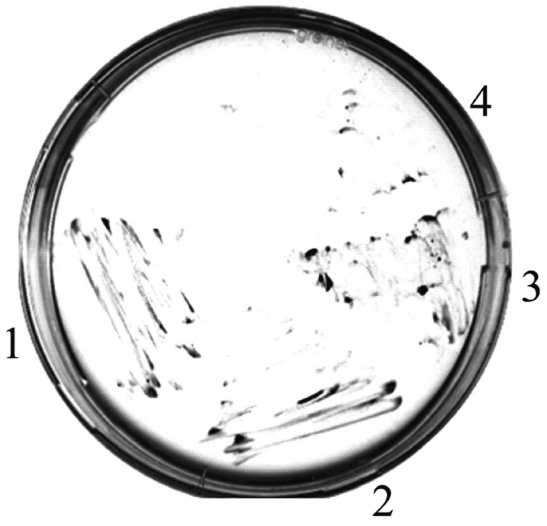

Figure 1. Blue/white screen for positive interaction partners of protein kinase CK2α. Yeast cells were transformed with pGBKT7-CK2α and pACT2-CK2β (1); p6BKT7-CK2α + clone H55 (2); pGBKT7-CK2α + clone H64 (3); pGBKT7-CK2α + pGADT7 (4) as a control. Yeast cells were grown under stringent conditions for 3 days. Positive interactions were verified by blue/white staining with x-gal.

Material and methods

Yeast-two-hybrid analysis and constructs. Yeast-two-hybrid screens with CK2α and CK2α' as baits were performed using the Matchmaker®-System from Clontech according to the manufacturer's instructions. Plasmids used for the screens were pGBKT7 that contained the respective CK2-subunits and a human testis cDNA library subcloned into pACT2. We used the following primers for cloning the motor domain and the non-motor domain of KIF5C into the vectors pGADT7-KIF5C-MD and pGADT7-KIF5C-NMD: KIF5C-MD forward: 5'-AGAGAGGAATT-CATGGCGGATTCCAGCCGA-3'; reverse: 5'-AGAGAGGAATTCTAGGTTCACAGAGACTG-TAT-3'; KIF5C-NMD forward: 5'-AGAGAGGAA-TTCGAACTGACAGCAGAAGAA-3'; reverse: 5'-AGAGAGGAATTCTTTCTGGTAGTGAGTG-GA-3'.

Yeast AH109 cells were co-transformed with the plasmids pGBKT7-CK2α or pGBKT7-CK2α' and either pGADT7-KIF5C-MD, pGADT7-KIF5C-NMD, pGADT7 or pACT2-CK2β and grown on SD-Leu/-Trp agar. Colonies were transferred to SD-Leu/-Trp/-His/-Ade agar. Finally, a blue/white screen was performed.

We used SKII(+) KIAA0531 from Prof. Nagase, Kazusa Research Center, Japan, as a template to clone the motor domain (1–335) and the non-motor domain (336–957) in frame into the *Eco*RI sites of

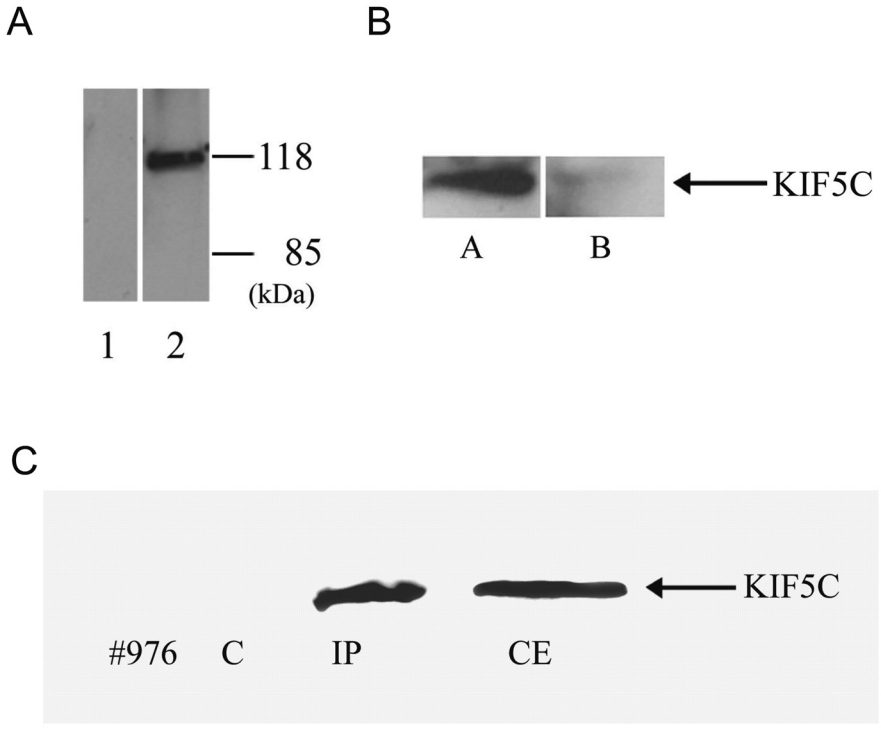

Figure 2. Characterization of serum #976 directed against KIF5C. (*A*) An aliquot from a cell extract from SH-SY5Y cells was run on a SDS-7.5% polyacrylamide gel. After electrophoresis proteins were transferred onto a PVDF membrane. The membrane was incubated either with pre-immune serum (1) or with serum #976 (2) followed by peroxidase-labeled goat anti-rabbit serum. Proteins were visualized by the ECL method. (*B*) Same as (*A*) but the rabbit serum #976 was either incubated in the presence of 10 mM peptide, which was used for the immunization (*B*) or in the absence of this peptide (*A*). (*C*) A cell extract from SH-SY5Y cells was either incubated with pre-immune serum (*C*) or with serum #976 (IP) and protein A Sepharose. Proteins were eluted from the precipitates and analyzed on a SDS-7.5% polyacrylamide gel. As a control an aliquot from the cell extract was loaded on the gel (CE). After electrophoresis, proteins were transferred to a PVDF membrane. The membrane was incubated with H1 antibody directed against KIF5C. Proteins were visualized by incubation with a peroxidase-labeled goat anti-mouse antibody and the ECL reaction.

pGADT7. All constructs were checked by DNA sequencing.

Cell culture. SH-SY5Y cells (human neuroblastoma cells, ATCC209) were maintained in Dulbecco's modified Eagle's medium (DMEM) HAM's F12 supplemented with 10% fetal calf serum (FCS). Neurons were grown in Neurobasal A medium supplemented with 2% B27, 1% Glutamax stock solution and 0.2% penicillin/streptomycin at 37°C in a 5% CO_2 atmosphere. Cells were grown to 75% confluence in 10-cm dishes in a 5% CO_2 atmosphere.

Antibodies and Western blot analysis. To detect CK2 we used rabbit anti-peptide sera #26 (α-subunit), #30 (α'-subunit) and #269 (β-subunit) [17, 18] and the monoclonal antibodies 1A5 [19] (α-subunit), 1AD9 (α and α' subunit) [20] and 6D5 (β-subunit) [21]. Rabbit serum #976 was raised against a peptide corresponding to amino acids 393–405 (CDNTPIIDNIAPV) of the KIF5C protein. We also used the mouse monoclonal antibody H1 (Chemicon) directed against KIF5C. Antibodies against KIF5C and CK2 from different species were useful for sequential immunoprecipitation and double staining in the immunofluorescence studies. The preparation of cell extracts, SDS-polyacrylamide gel electrophoresis, and blotting procedure were described earlier [22]. To detect proteins we used the Lumilight system (Roche Diagnostics).

Immunofluorescence. Cells were grown on coverslips until they were 50–70% confluent. Cells were fixed in 2% formaldehyde in PBS pH 7.4 for 15 min at 20°C and then washed with PBS pH 7.4 for 3×10 min. Cells were fixed with 3.7% formaldehyde and permeabilized with 0.5% Triton X-100. Cells were washed again three times with PBS for 10 min and then blocked with PBS containing 10% bovine serum albumin (BSA). They were then incubated with a primary antibody in the appropriate concentration for 1 h at room temperature in a humidified chamber. Cells were washed under the same conditions, and then incubated with the secondary antibody (ALEXA-fluor 488 or ALEXA-fluor 594) at room temperature for 1 h in a

Table 1. The catalytic subunits of protein kinase CK2 interact directly with the KIF5C motor domain.

BD vector	AD vector	Growth on –L, –W dropout media	Growth on –L, –W, –H dropout media
pGBKT7 CK2α	pGADT7	+	–
	pGADT7 $KIF5C_{1-335}$	+	+
	pGADT7 $KIF5C_{336-957}$	+	–
	pACT CK2 β	+	+
pGBKT7 CK2α′	pGADT7	+	–
	pGADT7 $KIF5C_{1-335}$	+	+
	pGADT7 $KIF5C_{336-957}$	+	–
	pACT CK2 β	+	+

dark, humidified chamber. Finally, cells were washed again with PBS (4×10 min). The coverslips were fixed with a drop of mounting medium and cells were analyzed under a fluorescence microscope.

Sucrose gradient analysis. Sucrose gradient analysis was performed as described [23]. Briefly, 2.5 mg cytoplasmic extract from SH-SY5Y cells was loaded on a 5–20 % linear sucrose gradient and centrifuged at 288 000 *g* in a Beckman SW41T rotor for 18 h. Thirteen fractions (~1 ml) were collected from the bottom to the top of the gradient. Aliquots of 0.5 ml of the fractions were used for immunoprecipitation and then loaded onto an SDS-10 % polyacrylamide gel to detect proteins in an immunoblot. We also used four protein markers, BSA (4.6 S), alcohol dehydrogenase (7.4 S), catalase (11.3 S) and β-galactosidase (16 S) in a parallel gradient for the determination of the S values.

Immunohistochemistry with bone marrow paraffin slices. Bone marrow slices on a coverslip were incubated twice with Xylol for 10 min, then with ethanol in decreasing concentration (10 min 100 % ethanol, 5 min 95 % ethanol, 1 min 70 % ethanol). After incubation with citrate buffer (citric acid 4.2 g/2 l, pH 6.0) and washing steps with water, the slices were incubated with PBS containing 0.01 % Triton X-100. Unspecific binding was avoided by incubation with blocking buffer (10 % FCS, 4 % milk powder in PBS). Slices were incubated with the primary antibody for 1 h. After intensive washing, slices were incubated with the secondary antibody overnight. After washing with water, slices were analyzed under a fluorescence microscope.

Surface plasmon resonance (SPR) spectrometry. SPR spectrometry was performed in a BIAlite upgrade system. Monoclonal goat anti-GST-antibodies (BIACORE, Freiburg, Germany) were immobilized on a sensor chip CM5 research grade (BIACORE) by amine coupling according to the manufacturer's protocol. The chip was equilibrated with running buffer (25 mM Tris-HCl pH 8.5, 100 mM NaCl, 100 mM KCl, 0.65 % CHAPS). The GST-KIF5C was bound to the immobilized antibodies in the measuring cell. Similarly immobilized GST in the reference cell served as a negative control. Subsequently, solutions containing purified CK2α' were passed over the chip (flow rate 5 µl/min), followed by application of running buffer. The analysis was carried out using the BIA evaluation software 3.1 (BIACORE).

Results

The catalytic subunits of CK2 interact with the kinesin heavy chain of KIF5C. To identify new binding partners of CK2, we performed two-hybrid screens with a cDNA library from human testis and CK2α or CK2α' cDNA as baits. In the course of these experiments we found a great number of positive clones. Out of 20 clones sequenced, the cDNA of 2 clones corresponded to nucleotides 762–1047 of the KIF5C gene. The interaction of both clones with the α-subunit in a yeast-two-hybrid re-screen under stringent growth conditions and after blue/white staining with x-gal is shown in Figure 1, and demonstrate that both interacted with CK2α (2 and 3). As a positive control we show the interaction of CK2α with CK2β (1), and as a negative control the one of CK2α with the empty vector (4). To confirm the binding region on the KIF5C polypeptide chain, we used two additional KIF5C constructs. pGADT7-KIF5C-MD corresponded to the motor domain of KIF5C (amino acid 1–335) and pGADT7-KIF5C-NMD to the non-motor domain of KIF5C (amino acid 336–957). Under highly stringent conditions, *i.e.*, in a triple dropout medium, both catalytic subunits of CK2 bound to the motor domain but not to the non-motor domain of KIF5C, confirming and extending our initial yeast-two-hybrid analysis and the sequencing results (Table 1). Fur-

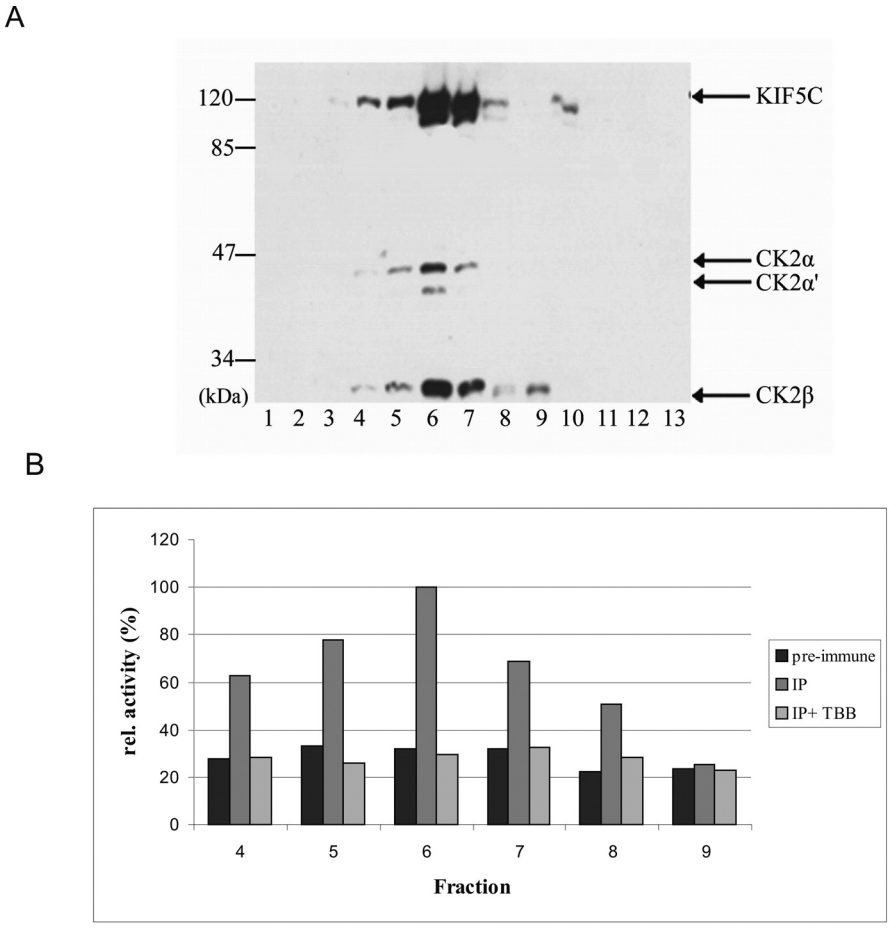

Figure 3. Sucrose density gradient analysis of a cell extract from SH-SY5Y cells for KIF5C and CK2 subunits. (*A*) A cell extract from SH-SY5Y cells was loaded on a linear 5–20 % sucrose density gradient and centrifuged for 16 h at 288 000 *g*. Fractions were collected from the bottom of the tube and half of the amount in each fraction was analyzed on a SDS-12.5 % polyacrylamide gel. Proteins were transferred to a PVDF membrane. The membrane was incubated with antibody H1 to detect KIF5C, with monoclonal antibody 1AD9 to detect CK2α and CK2α' and monoclonal antibody 6D5 for CK2β. Protein bands were visualized by the ECL method. The molecular weight marker is indicated on the left. (*B*) The other half of each fraction was preincubated with protein A-Sepharose. The supernatants were immunoprecipitated with serum #976 and protein A-Sepharose. After washing the immune precipitates were divided into two equal aliquots and incubated with [γ-^{32}P]ATP and the CK2-specific substrate peptide either in the presence or in the absence of the CK2 inhibitor TBB. Incorporation of ^{32}P into the substrate peptide was measured by Čerenkov radiation. The activity in the immunoprecipitate in fraction 6 was set 100 %; other activities were calculated in relation to this 100 % value.

thermore, we also tested CK2α' for its interaction with the N- and C-terminal domains of KIF5C. As shown in Table 1, CK2α' showed the same binding behavior as CK2α. Thus, according to these yeast-two-hybrid-experiments, KIF5C seems to bind to both catalytic subunits of CK2.

A mouse monoclonal antibody against KIF5C is commercially available. For co-immunoprecipitation experiments and for double-staining experiments in immunofluorescence studies a rabbit antibody against KIF5C was required. We screened the polypeptide chain for a sequence that is unique for KIF5C. A peptide corresponding to amino acids 495–503 from the KIF5C protein that was exclusively present in KIF5C and not in KIF5A and KIF5B was synthesized, coupled to hemocyanin and then used to immunize a rabbit. The serum, which was named #976, was tested in a Western blot analysis using cytoplasmic extracts from several different cell lines from various tissues for the presence of KIF5C. Two cell lines, SH-SY5Y, a human neuroblastoma cell line (Fig. 2A), and NSC-34, a hybrid spinal cord neuroblastoma cell line (data not shown), were positive for KIF5C. No protein band was detected by the pre-immune serum (lane 1), whereas the immune serum clearly detected a protein band migrating at the calculated molecular weight (lane 2). Next, we repeated the Western blot analysis with serum #976 in the presence or absence of the peptide that was used for the immunization of the rabbit. As shown in Figure 2B, in the presence of the peptide (lane B), we could not detect a protein band, whereas a protein band was clearly detected in the

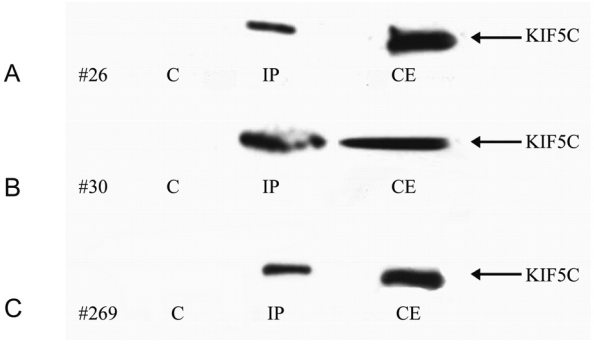

Figure 4. Co-immunoprecipitation analysis of KIF5C and the individual subunits of CK2. A cell extract form SH-SY5Y cells was pre-incubated with protein A-Sepharose. The supernatant of this pre-incubation step was incubated with serum #26, #30 or #269 against the CK2 subunits. The immunoprecipitates were analyzed on a SDS-7.5 % polyacrylamide gel. Proteins were transferred to a PVDF membrane. KIF5C was detected with antibody H1, a peroxidase-coupled secondary antibody and the ECL method. C, serum #26 alone, serum #30 alone, or serum #269 alone; IP, co-immunoprecipitate; CE, total cell extract.

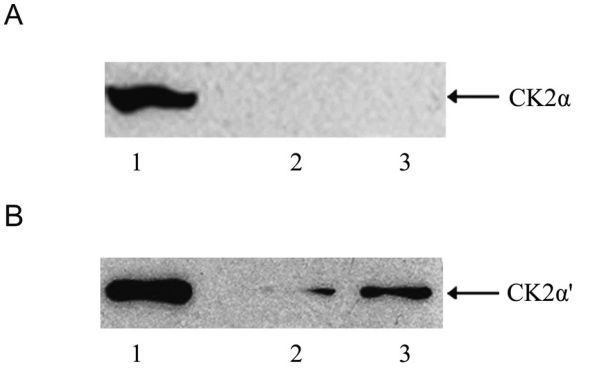

Figure 5. Pull-down experiments of KIF5C with CK2α and CK2α'. GST-tagged KIF5C was coupled to GSH-Sepharose and then incubated with CK2α or CK2α' (lanes 3). As a control GSH-Sepharose was incubated with CK2α (A lane 2) and CK2α' (B lane 2). Lanes 1 show aliquots of CK2α and CK2α'. Proteins eluted from the affinity columns were analyzed on an SDS-12.5 % polyacrylamide gel, and transferred to a PVDF membrane. CK2α was detected with antibody #26, CK2α' was detected with antibody #30.

absence of the peptide (lane A), showing the specificity of the serum. To test whether serum #976 can be used for immune precipitation of KIF5C from a cytoplasmic extract of SH-SY5Y cells, the immune precipitate was analyzed on an SDS polyacrylamide gel. Lane (IP) in Figure 2C shows that antibody #976 immunoprecipitated KIF5C, whereas the pre-immune serum is negative (lane C). Lane (CE) shows KIF5C in a total cell extract as a control. Thus, antibody #976 can be used for Western blot analysis as well as for immunoprecipitation.

KIF5C co-sedimented with CK2 on a sucrose density gradient. To further analyze a possible KIF5C/CK2 interaction, an SH-SY5Y cell extract was applied to a 5–20 % sucrose density gradient. Aliquots from the gradient were collected and analyzed on a 10 % polyacrylamide gel. Proteins were visualized with rabbit serum #976 against KIF5C, the mouse monoclonal antibody 1AD9 that recognizes CK2α and CK2α', and mouse monoclonal antibody 6D5 that is directed against CK2β. As shown in Figure 3A, KIF5C and the CK2 subunits were mainly found in fractions 5–7. Thus, on a sucrose density gradient, KIF5C co-sedimented with all three subunits of CK2. To analyze individual fractions of the gradient that contained CK2 and KIF5C for a KIF5C-associated kinase activity, we immunoprecipitated KIF5C with serum #976. The immunoprecipitates were used to phosphorylate a CK2-specific substrate peptide. As shown in Figure 3B, fractions 5–7 from the sucrose density gradient exhibited the highest CK2 activity, showing that immunopurified KIF5C was associated with a CK2 activity. To further confirm that this activity was specific for CK2, we repeated the experiment described above in the presence of the CK2-specific inhibitor tetrabromobenzotriazol (TBB) [24]. In this case the kinase activity was reduced to nearly background levels (Fig. 3B). Precipitates with protein A-Sepharose were used as a control. These results support the observation that CK2 co-sedimented with KIF5C, and furthermore show that immunoprecipitated KIF5C is associated with CK2.

To analyze the association between CK2 and KIF5C, we performed co-immune precipitation experiments. A cell extract of SH-SY5Y cells was incubated either with serum #26 (specific of CK2α), serum #30 (specific for CK2α') [17] or serum #269 (specific for CK2β) [18]. The immunoprecipitates were analyzed on an SDS-10 % polyacrylamide gel. After transfer to a PVDF membrane, the membrane was incubated with antibody #976 against KIF5C. As shown in Figure 4A–C, KIF5C was co-immunoprecipitated with all three CK2-specific antibodies. A co-immunoprecipitation reaction was also performed in the opposite direction using a KIF5C-specific antibody. In this case all three CK2 subunits were detected by Western blotting (data not shown). These results indicate that KIF5C binds either to all three subunits of CK2 or to the CK2 holoenzyme.

To define which CK2 subunit binds directly to KIF5C, we performed pull-down experiments with GST-tagged KIF5C protein and purified CK2 catalytic subunits. Bound subunits were then analyzed on SDS-polyacrylamide gels. As shown in Figure 5B lane 3, CK2α' bound to KIF5C, whereas CK2α did not (Fig. 5A, lane 3), indicating that KIF5C bound to the

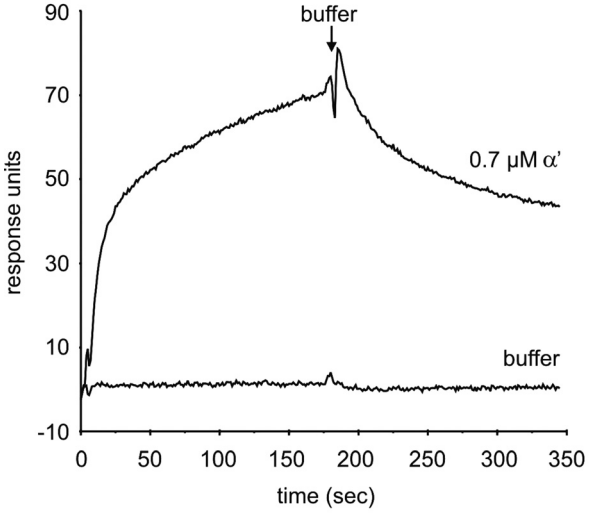

Figure 6. Sensorgram of the interaction of KIF5C with the CK2α' subunit. Monoclonal goat anti-GST antibodies were immobilized on a sensor chip CM5. GST-KIF5C was bound to the immobilized antibodies in the measuring cell. Buffer or defined concentrations of CK2α' were passed over the chip (association phase). This was followed by application of running buffer (dissociation phase). The sensorgram shows the SPR difference (in response units) between the measuring cell and the reference cell containing immobilized GST only.

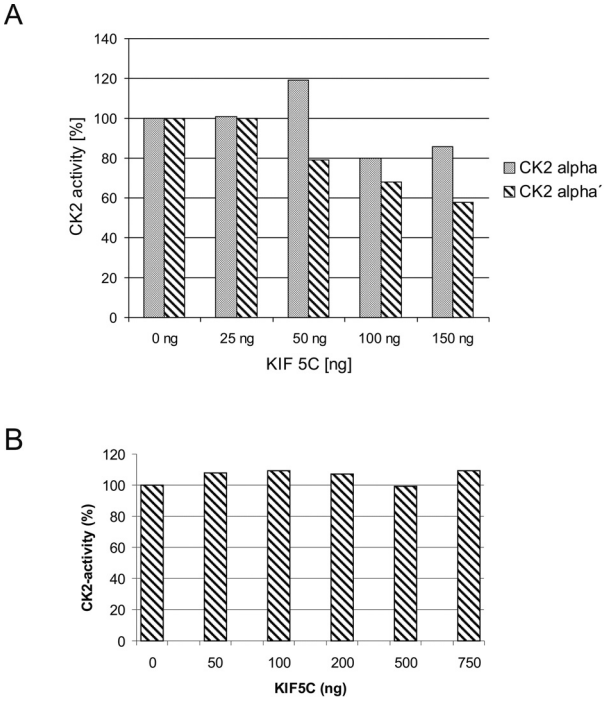

Figure 7. Influence of KIF5C on the kinase activity of the CK2 holoenzyme, of CK2α and of CK2α'. Increasing amounts of KIF5C were incubated with CK2α, CK2α' (*A*) or the CK2 holoenzyme (*B*) together with [γ-^{32}P]ATP and the CK2-specific substrate peptide RRRDDDSDDD. Incorporation of ^{32}P into the substrate peptide was measured by Čerenkov radiation. Incorporation of phosphate in the absence of KIF5C was set 100%.

holoenzyme *via* the CK2α' subunit and/or to CK2α' alone. Lanes 1 show aliquots of CK2α (A) or CK2α' (B). In another approach, we performed an SPR spectrometry analysis with KIF5C and the two catalytic CK2 subunits. As shown in Figure 6, KIF5C bound to the CK2α' subunit. No specific interaction between KIF5C and CK2α was detected (data not shown). Thus, the biosensor measurements confirm the results obtained by pull-down assays. Next, we asked whether KIF5C had an influence on the kinase activity of the catalytic subunits or the CK2 holoenzyme. CK2α, CK2α' or the CK2 holoenzyme was incubated with increasing concentration of purified KIF5C and [γ-^{32}P]ATP and the CK2-specific substrate peptide with the sequence RRRDDDSDDD as described earlier [25]. Incorporation of ^{32}P into the substrate peptide was measured by Čerenkov radiation, where the phosphate incorporation in the absence of KIF5C was set 100%. As shown in Figure 7A, increasing concentrations of KIF5C led to a reduction of the kinase activity of CK2α' in a dose-dependent manner and, to a much slighter degree, to a reduction of the activity of CK2α. On the other hand, even higher concentrations of KIF5C had no influence on the kinase activity of the CK2 holoenzyme (Fig. 7B).

Co-localization of KIF5C with the CK2 subunits. There are controversial data about the subcellular localization of the CK2 subunits, which might be due to the fact that the subcellular localization of CK2 reflects a dynamic process. The subcellular localization of individual CK2 subunits might also reflect an interaction with one particular cellular partner molecule. Therefore, we analyzed the subcellular localization of the CK2α, α' and β and of KIF5C in the neuroblastoma cell line SH-SY5Y. Cells were incubated either with serum #26, serum #30 and serum #269 (all from rabbits) to detect the individual CK2 subunits, or with mouse monoclonal antibody H1 specific for KIF5C. As shown in Figure 8, KIF5C was mainly localized in the cytoplasm. CK2α was mostly found in the nucleus, whereas CK2α' and CK2β were distributed in the cytoplasm and in the nucleus. The overlay shows that all three CK2 subunits in the cytoplasm co-localized, at least in part, with KIF5C. KIF5C is mainly present in motor neurons. Since bone marrow is enriched for motor neurons, we analyzed a co-localization of CK2 subunits and KIF5C in bone marrow from mice. Paraffin-embedded bone marrow slices were incubated with antibody H1 against KIF5C, antibody #26 against CK2α, and #30 against the α'-subunits of CK2. The staining pattern for CK2α, CK2α', and KIF5C was nearly the same (Fig. 9), arguing for a co-localization of the catalytic

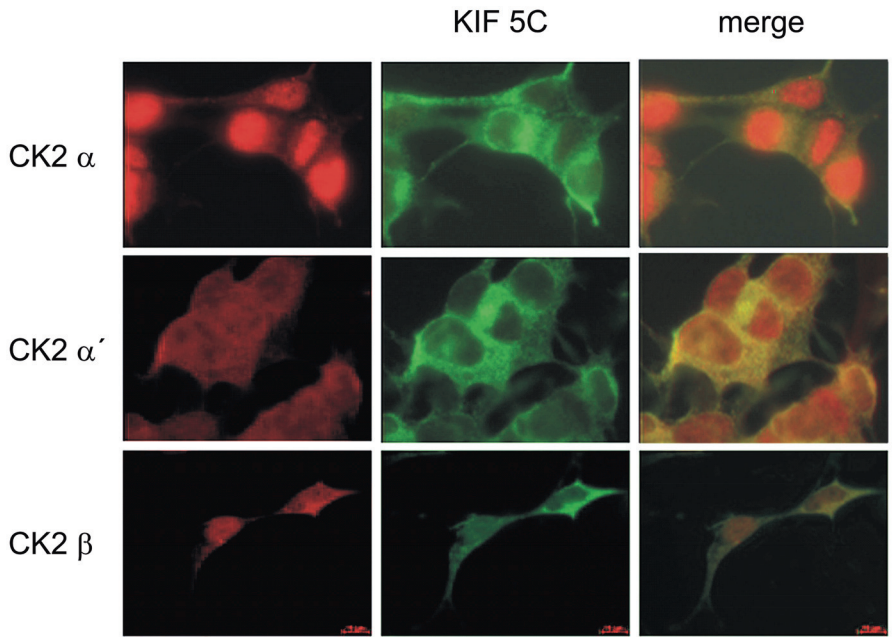

Figure 8. Subcellular localization of KIF5C and CK2 subunits in SH-SY5Y cells. Cells were seeded on coverslips, fixed with 3.7 % formaldehyde and permeabilized with 0.5 % Triton X-100. Cells were either incubated with antibody H1 (KIF5C) or antibodies #26 (CK2α), #30 (CK2α′) or #269 (CK2β). As secondary antibodies, we used ALEXA-fluor 488 or ALEXA-fluor 594. Immunofluorescence was analyzed with a Zeiss Axiovert microscope. Magnification 1:1000.

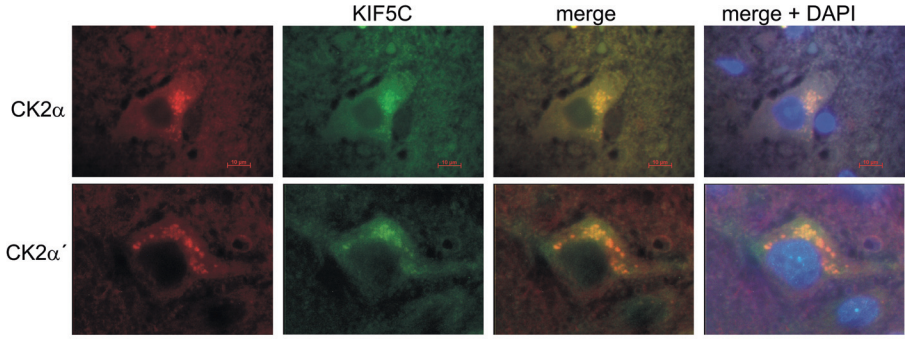

Figure 9. KIF5C and CK2 subunits in bone marrow sections from mice. Paraffin sections of bone marrow from mice were deparaffinized and then incubated with antibody H1(KIF5C), antibody #26 (CK2α) or the antibody #30 (CK2α'). After washing, slices were incubated with goat anti-mouse ALEXA-fluor 488 or goat anti-rabbit ALEXA-fluor 594. Fluorescence was analyzed with a Zeiss Axiovert microscope. Magnification ×400.

subunits of CK2 and KIF5C proteins also in bone marrow.

Under certain conditions an elevated level of CK2 has been shown for neurons [26]. Therefore, we analyzed the subcellular localization of CK2 subunits and KIF5C in primary hippocampus neurons by double immunofluorescence labeling. We used the same antibodies as described above for the analysis of CK2 subunits and KIF5C in bone marrow cells. CK2α was mainly found in the nucleus whereas CK2α' and KIF5C were mainly expressed in the cytoplasm (Fig. 10). Merging the fluorescence signals revealed a co-localization of mainly CK2α' and KIF5C. Thus, these results show that KIF5C interacts with the catalytic subunits of CK2 *in vivo* and *in vitro*. Under certain conditions KIF5C seems to have a preference for binding to CK2α'.

Discussion

Research on protein kinases, their regulators as well as their substrates plays an increasing role in understanding the proteome. CK2, already known for more than 50 years, has recently attracted much interest. The number of known substrates for this kinase is increasing rapidly and it is clear that CK2 is implicated in basic processes such as DNA replication, transcription and translation [27, 28]. Furthermore, CK2 plays a role in regulating intracellular transport such as nuclear import [29–33]. Thus, CK2 is believed to be a protein kinase that is especially required for cell cycle progression and for proliferation of cells. There are some findings indicating that CK2 might have neural functions. CK2 is much more abundant in brain than in any other tissue [34–36], which corresponds with the high activity of CK2 in brain [37, 38]. CK2 is present in all brain regions studied, such as cortex, septum, hippocampus, cerebellum and spinal cord [39,

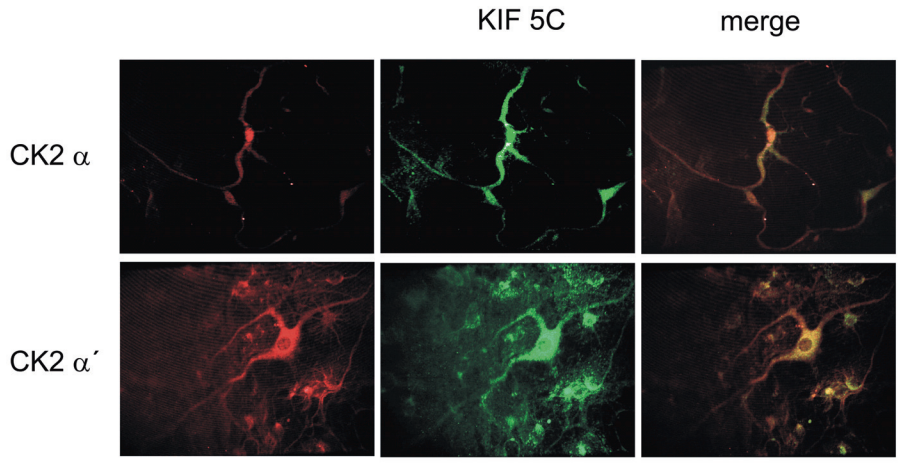

Figure 10. KIF5C and CK2 subunits in primary neurons from the hippocampus. Neurons were fixed with ice-cold methanol, permeabilized with Triton X-100 and then incubated with antibodies #26 (CK2α) or #30 (CK2α') or antibody H1 (KIF5C). As a secondary antibody we used goat anti-mouse ALEXA-fluor 488 or goat-anti-rabbit ALEXA-fluor 594. Fluorescence was analyzed with a Nipkow disc-based laser-scanning confocal microscope comprising a confocal head (QLC-100, VisiTech Int., KK) attached to an upright microscope (Eclipse E600, Nikon, Japan).

40]. Both, CK2α and CK2α' seem to play a role during rat brain development [34, 41]. CK2α was found to appear within mature rat neurons at the time of dendritic maturation and synaptogenesis [41]. It was supposed that the high cytosolic levels of CK2 might contribute to help phosphorylation of the microtubule-associated protein 1B (MAP-1B) during neurite outgrowth [26]. In neuroblastoma cells in which the activity of CK2 was deleted through antisense technology, neuritogenesis and cell polarity were inhibited [42].

Using a yeast-two-hybrid approach with CK2α or CK2α' as a bait, we identified a member of the kinesin-1 family, KIF5C, as a binding partner for both catalytic CK2 subunits. These findings were confirmed by co-sedimentation of KIF5C and CK2 on a sucrose density gradient and by co-immunoprecipitation. These later experiments indicate that the holoenzyme, consisting of CK2α-, CK2α'- and the CK2β-subunits was bound to KIF5C. However, pull-down binding experiments with individual subunits revealed that KIF5C only binds to CK2α' and not to CK2α and CK2β. Furthermore, in a very sensitive SPR spectrometry analysis, only the CK2α' bound specifically to KIF5C, confirming the pull-down experiments. Moreover, KIF5C only significantly inhibited the kinase activity of CK2α' and not that of the holoenzyme. The co-immunoprecipitation results can be explained by an interaction of KIF5C with CK2α' in its free forms, as well as in the holoenzyme. In a similar approach using the CK2α' subunit in a two-hybrid screen, a cDNA coding for the ubiquitin-conjugating enzyme UBC3B were identified by Semplici et al. [43]. Further experiments showed that UBC3B was also phosphorylated by CK2 *in vivo* and *in vitro*. Recently, we reported that KIF5C was phosphorylated by CK2 *in vivo* and *in vitro*. Furthermore, we found that KIF5C was phosphorylated by holoenzymes composed of CK2α/CK2β and CK2α'/β as well as by CK2α' alone, but not by CK2α alone [25]. Serum treatment of quiescent mouse fibroblasts induced CK2α' mRNA expression. Moreover, ectopic CK2α' expression together with Ha-ras led to foci formation of rat primary embryo fibroblasts [44], indicating a role of CK2α' in cell transformation. These various data support the idea of individual roles of the different CK2 subunits.

In *S. cerevisiae*, it was shown that CK2α' has properties that are characteristic for the CK2 holoenzyme with some exceptions. CK2α' is not inhibited by heparin and stimulated by polyamines, and it shows differences in the substrate specificity [45]. Furthermore, CK2α phosphorylates the β-subunit more rapidly and to a higher extent than CK2α' [46]. Recently, it was shown that superoxide dismutase SOD1 binds specifically to CK2α' thereby inhibiting its kinase activity. Thus, according to the data presented here, KIF5C is another protein that shows a preference for CK2α' at least in neurons, motor neurons and binding assays *in vitro*.

Many authors have investigated the subcellular localization of CK2, but the results are more confusing than illuminating [37, 39, 47, 48]. The enzyme has been found throughout the cell from the nucleus to the plasma membrane [29]. It was shown that the nuclear import and export of the CK2 subunits are regulated independently of each other, which results in rapid changes of their intracellular distribution [49]. In accordance with earlier studies in neuroblastoma cells, we found CK2α mainly in the nucleus, and CK2α' and CK2β mostly in the cytoplasm [26, 50]. KIF5C was exclusively found in the cytoplasm. Since CK2α and CK2β do not bind to KIF5C, these data support our binding experiments where KIF5C bound only to the α'-subunit of CK2. A cytoplasmic localization of an EYFP-tagged KIF5C has recently been shown in

transfected COS-7 cells, which is in agreement with our results with endogenous KIF5C in neurons and neuroblastoma cells [51]. Furthermore, it should be noted that a microtubule-binding site was found in the motor domain of KIF5C between amino acids 1 and 335. CK2 was also found to be associated with microtubules [52] and tubulin [17]. One might speculate that KIF5C transports CK2 to specific proteins at the cytoskeleton where it may phosphorylate cytoskeletal proteins such as myosin heavy and light chains [53], troponin-T, β-tubulin, tau protein [54], MAP-1B protein [55] and/or dynein [56].

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