Specific binding of protein kinase CK2 catalytic subunits to tubulin

Michael Faust, Norbert Schuster, Mathias Montenarh*

Medical Biochemistry and Molecular Biology, University of Saarland, Building 44, D-66424 Homburg/Saar, Germany

Received 21 September 1999; received in revised form 26 October 1999

Abstract Protein kinase CK2 is composed of two regulatory β-subunits and two catalytic α- or α′-subunits. To analyse these subunits individually we generated antibodies against unique peptides derived from the α-, α′- and β-subunit. Immunofluorescence studies with these antibodies revealed the presence of all three CK2 subunits in the cytoplasm and weakly in the nucleus with strong signals around the nuclear membrane. Double staining experiments revealed a co-localisation of all three subunits with tubulin. A direct association between the CK2 α- and the α′-subunit and tubulin was confirmed by co-immunoprecipitation experiments as well as by Far Western analysis. There was no binding of the CK2 β-subunit to tubulin. Thus, with tubulin we have identified a new binding partner specific for the catalytic subunits of CK2.

Key words: CK2 subunit-specific antibody; Immunofluorescence; Tubulin; Co-localisation; Protein-protein interaction

1. Introduction

Protein kinase CK2 is a ubiquitously expressed protein kinase which is composed of two catalytic α′- or α-subunits and two regulatory β-subunits [1,2].

This enzyme catalyses the phosphorylation of a great number of substrates which are implicated in transcription, signal transduction processes, growth regulation, various steps of development and in the formation of cellular shape and architecture [2]. Substrates of CK2 include cytoskeletal proteins such as myosin heavy and light chains [2], troponin-T, β-tubulin, tau protein [3], Mab-1B protein [4] and dynein [5]. Substrates of CK2 are localised in the cytosol, in the nucleus and under certain circumstances also at the nucleosomes, at the centrosomes [6], to the spindle apparatus in dividing cells [7], at the nuclear matrix [8,9] and in the nucleoli [10]. Phosphorylation of these proteins affects their assembly and dynamics. CK2 is not only implicated in the expression of a distinct cell type but also in differentiation and movement of cells. CK2 itself has been found to be present in the nucleus, cytosol, in microsomal and mitochondrial fractions [11,12] and in the extracellular matrix [13]. In addition to the interaction between an enzyme and a substrate there are accumulating data that CK2 as a holoenzyme or that the individual subunits of CK2 bind to a number of cellular and viral proteins (for review see: [1]). Among these proteins is the growth suppressor p53 which binds to the CK2 β-subunit [14,15] and the $p21^{WAF1}$ protein, which is transactivated by p53 [16]. In both cases binding of these proteins to CK2 leads to a reduction in the activity of CK2 [16,17] indicating that these two proteins are negative regulators of the activity of CK2. The nucleolar protein NOPP140 also binds to the β-subunit of CK2 and it is suggested that this interaction may be implicated in the cytoplasmic/nuclear transport of the β-subunit [18]. In addition also mos and the A-raf kinase, proteins which are involved in signal transduction bind to the β-subunit of CK2 [19,20]. Also binding partners for the α-subunit of CK2 such as nucleolin [10] and PP2a [21] are known whereas only little is known about proteins binding to the α′-subunit of CK2.

Studies on the subcellular localisation were mainly performed with antibodies raised against the CK2 holoenzyme or against the α- or β-subunit. Although the α- and the α′-subunits are highly homologous to each other each subunit is encoded by a separate gene [22] and there are unique regions on the polypeptide chains of CK2α and α′. There is ample evidence that different tissue types may express the α- and the β-subunits at varying levels (for review see: [23]) whereas little is known about the expression of the α′-subunit. We now have generated antibodies against individual peptides of the CK2 α-, α′- or β-subunit. With these antibodies we localised all three subunits in mouse embryo fibroblasts in the cytosol and in the nucleus with an accumulation around the nuclear membrane. It turned out that this immunostaining was very similar to the staining that was observed for tubulin. Co-immunoprecipitation studies and in vitro binding studies revealed that the α- and α′-subunit bound to tubulin whereas there is no direct binding of tubulin to the regulatory β-subunit of CK2.

2. Materials and methods

2.1. Cell culture

Mouse embryo fibroblasts (MEF1229) [24] and RKO cells [25] were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS). Cells were grown to 75% subconfluence in 10 cm dishes in a 5% CO_2 atmosphere.

2.2. Preparation of cell extracts

Cells were harvested, washed 3× with phosphate buffered saline (PBS), pH 7.4 and resuspended in lysis buffer (100 mM Tris-HCl, pH 9.0, 100 mM NaCl, 0.5% (v/v) NP40, 1% Trasylol). Proteins were extracted for 1 h on ice. Cell debris were eliminated by centrifugation (4°C, 30 min, 13 000×*g*).

2.3. 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 permeabilised with 0.2% Triton X-100 containing 1% normal goat serum (NGS) in PBS for 5 min on ice. Cells were washed again with PBS containing 1% NGS 3 times for 10 min and then incubated with a primary antibody in the appropriate concentration for 1 h at

*Corresponding author. Fax: (49) (6841) 166027.
E-mail: tm13mm@rz.uni-sb.de

PII: S0014-5793(99)01492-1

room temperature in a humidified chamber. Cells were washed under the same conditions. Cells were incubated with the secondary antibody (FITC or TRITC conjugated) at room temperature for 1 h in a dark, humidified chamber. Finally, cells were washed again with PBS (4×10 min). The coverslips were fixed with a drop of mounting media and analysed under a fluorescence microscope.

2.4. Far Western blot analysis

Proteins dissolved in sodium dodecyl sulfate (SDS) buffer (130 mM Tris-HCl, pH 6.8, 0.02% bromophenol blue (w/v), 10% 2-mercaptoethanol, 20% glycerol (v/v), 4% SDS were separated in a SDS-polyacrylamide gel, renatured in 10× PBS for 2 h on ice, then the refolded proteins were transferred onto a PVDF Western blotting membrane (Boehringer Mannheim) in a buffer containing 20 mM Tris-HCl, 150 mM glycine, pH 8.9. The membrane was blocked for 1 h in binding buffer (0.05% (v/v) Tween 20 in PBS, pH 7.4) with 5% dry milk. The probe (tubulin) with a final concentration of 1 μg/μl was diluted in binding buffer with 1% dry milk and incubated for 1 h at 4°C. The membrane was washed in PBS and incubated for 1 h with an antibody against the probe which was dissolved in binding buffer with 1% dry milk in a dilution of 1:1000. The blot membrane washed in binding buffer was decorated with the secondary antibody and assayed with the ECL system (Amersham, Braunschweig, Germany) according to the manufacturer's instructions.

2.5. Immunoprecipitation

For immunoprecipitation we use polyclonal sera (serum #26, #30, #32) raised against C-terminal peptides from protein kinase CK2 α- (CTPSPLGPLAGSP), CK2 α′- (CEQSQPCADNAVLSSGLTAAR) or CK2 β-subunit (CSNFKSPVKTIR), respectively, and monoclonal anti-α-tubulin antibodies (Sigma). A protein A/G-sepharose mixture was preincubated for 1 h with 50 μl of the different sera or with 10 μl (1 μg/μl) of the monoclonal anti-α-tubulin antibody and washed three times with PBS, pH 7.4. One miligram cell extract or alternatively 10 μg purified proteins were preincubated with a mixture of protein A- and protein G-sepharose (Pharmacia) to remove unspecific binding proteins. The supernatant was applied to the preincubated sepharose-antibody-matrix and incubated for 1 h. The supernatant was removed and the antibody-matrix washed three times with NET buffer with 0.5% (v/v) NP40. The immune complex was subjected to SDS-polyacrylamide gel electrophoresis, followed by Western blotting.

2.6. Purification of CK2 holoenzyme

Recombinant CK2 holoenzyme cloned in a bicistronic vector [26] was expressed in *Escherichia coli* BL21 (DE3) and the holoenzyme was purified according to a protocol published by [27]. The bacterial pellet was lysed and after incubation on ice for 10 min, the lysate was sonicated two times and centrifuged (10 000×*g*, step 1). The pellet was resuspended in buffer P1500 (20 mM Tris-HCl, pH 8.0, 7 mM 2-mercaptoethanol, 1 mM phenylmethyl sulfonylchloride (PMSF) and 1.5 M NaCl). Proteins were extracted overnight, pooled with the supernatant from the first step, and dialysed against buffer P300 (20 mM Tris-HCl, pH 8.0, 7 mM 2-mercaptoethanol, 1 mM PMSF and 300 mM NaCl). The lysate was loaded onto a P11 column pre-equilibrated with buffer P300 and eluted with a linear gradient from 0.3–1.5 M NaCl. Fractions containing active holoenzyme were dialysed against buffer P300, concentrated and loaded onto a Superose-6 column (Pharmacia). After gel filtration the peak fractions were collected.

2.7. Expression and purification of bacterially expressed proteins

The three different subunits (α, α′ and β in pGS) of the protein kinase CK2 were transformed into *E. coli* strain BL21 (DE3). Clones were incubated overnight in 50 ml LB-medium. One liter LB-medium was inoculated with the overnight-culture, grown to early log phase and induced with 1 mM isopropyl-β-D-thiogalactoside (IPTG) at 30°C for 6 h. Cells were harvested by centrifugation and resuspended in 6 M guanidine hydrochloride, 0.1 M sodium phosphate, pH 8.0 and lysed at 4°C overnight. The lysate was cleared by centrifugation and loaded onto a prewashed Ni^{2+}-chelate agarose column and incubated for 1 h at room temperature. The column was washed with 10 volumes of lysis buffer, followed by 10 volumes of lysis buffer pH 6.0, and lysis buffer pH 8.0 containing 20 mM imidazole. Proteins were eluted with lysis buffer containing 300 mM imidazole and subsequently dialysed overnight against dialysis buffer A (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 5 mM $MgCl_2$, 0.1% Tween 20).

3. Results

In order to analyse the individual subunits of CK2 we generated a rabbit serum against a peptide of 13 amino acids derived from the unique region of the α-subunit from amino acid 360 to 371 (serum #26) (Fig. 1A). An other antibody (serum #30), which was also raised in rabbits, is directed against a peptide of 21 amino acids corresponding to the unique sequence of the α′-subunit from amino acid 330 to 349 (Fig. 1A). Rabbit serum #32 was raised against a peptide corresponding to the 10 last amino acids of the β-subunit (Fig. 1A). In order to test the specificity of the newly generated antibodies individual subunits of CK2 were expressed in bacteria and the proteins separated on an SDS polyacrylamide gel followed by a transfer onto a PVDF membrane. The membrane with the α-subunit was either incubated with serum #26 or as a control with monoclonal antibody 1AD9 [28] directed against a common region of the α- and α′-subunit. The membrane containing the α′-subunit was either incubated with serum #30 or with monoclonal antibody 1AD9. The third membrane containing the β-subunit of CK2 was incubated either with serum #32 or with monoclonal antibody 6D5 [29] as a control. Fig. 1B shows that all three sera reacted with the corresponding subunits comparable to the monoclonal antibodies used as controls. There was no cross-reactivity of the antibody against the α′-subunit with the α-subunit or vice versa (data not shown).

Next, we wanted to know whether the newly generated antibodies would react with the proteins in a cell extract and whether they would also precipitate the CK2 holoenzyme. Cell extracts from RKO cells [25] were incubated with either serum #26, serum #30 or serum #32. Immunoprecipitates were analysed on an SDS polyacrylamide gel followed by a Western blot analysis using a mixture of monoclonal antibodies 1AD9 and 6D5. Fig. 1C shows that all three sera immunoprecipitated the CK2 holoenzyme, although the amount of the α- or the α′-subunit in the immunoprecipitates with serum #26 or serum #30 varied. In the serum #26 immunoprecipitate the α′-subunit is hardly detectable (lane 2) whereas in the serum #30 immunoprecipitate there is a great excess of α′-subunit compared to the α-subunit. Thus, we have shown that the newly generated antibodies react with the individual subunits and moreover they immunoprecipitate the CK2 holoenzyme from a mammalian cell extract. To study the subcellular distribution of individual subunits of CK2 we used primary mouse embryo fibroblasts in order to avoid any cellular influences by cell transformation, viruses or other factors. Cells were grown to subconfluence, fixed and incubated with the sera against the individual subunits of CK2, followed by a rhodamine labelled goat anti-rabbit serum. As shown in Fig. 2A–C all three subunits of CK2 were localised in the cytoplasm and in the nucleus with an accumulation around the nuclear membrane. This subcellular distribution resembled the subcellular localisation described for tubulin [30]. Therefore, we performed a double labelling experiment. Cells were incubated with serum #26 directed against the α-subunit of CK2 followed by a TRITC labelled goat anti-rabbit serum. Then, cells were incubated with a mouse anti-α-tubulin antiserum followed by an FITC labelled goat anti-mouse serum. As shown in Fig. 2D,E both proteins co-localised in the cell around the nuclear membrane. A very similar co-localisation was shown for the α′- and for the β-subunit of CK2 (data not

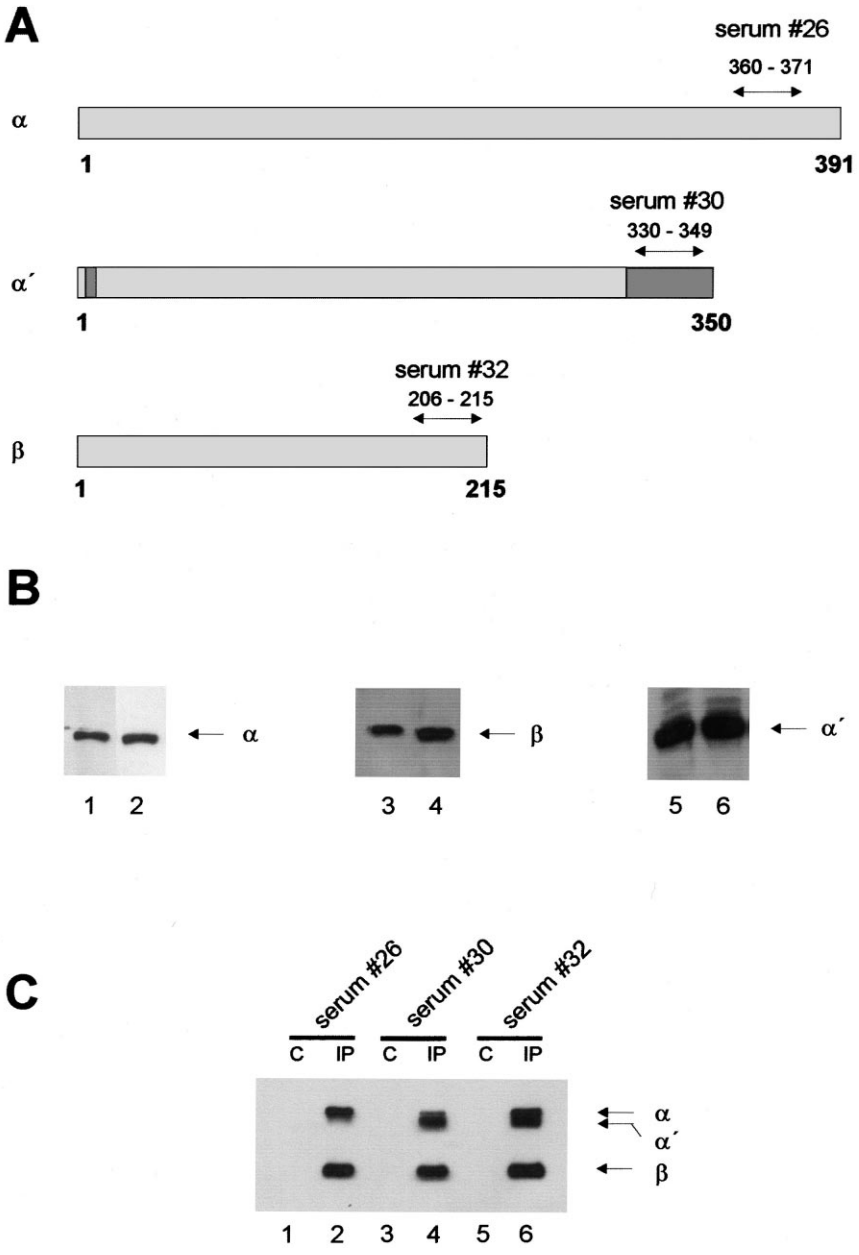

Fig. 1. Characterisation of newly-generated sera against CK2 α- (serum #26), α′- (serum #30) and β-subunit (serum #32). A: Location of selected epitopes on the polypeptide chain of CK2 subunits. B: Western blot analysis of purified recombinant subunits of CK2 with serum #26 (lane 2), #30 (lane 4) and #32 (lane 6). As a control the membrane was decorated with established monoclonal antibodies 1AD9 against the α- and α′-subunit (lanes 1 and 5) and 6D5 against the β-subunit (lane 3). C: Immunoprecipitation experiment with serum #26 (lane 2), #30 (lane 4) and #32 (lane 6). CK2 subunits were precipitated from 1 mg cell extract. Immunoprecipitates were separated by SDS-polyacrylamide gel electrophoresis and transferred onto a PVDF-membrane. CK2 was detected with a mixture of monoclonal antibodies 1AD9 and 6D5. C: control precipitate without antibody.

shown). A control experiment with an antibody against actin gave a different staining pattern for the CK2 subunits and for actin indicating that the CK2 subunits were not co-localised with actin (data not shown).

Having shown the co-localisation of the CK2 subunits with α-tubulin by immunofluorescence we next asked whether we could show an association of these proteins with biochemical methods. Therefore, we tried to co-immunoprecipitate CK2 complexes with tubulin from eukaryotic cell extracts. Mouse embryo fibroblasts were extracted and the cell extract incubated with serum against the α-, α′- or the β-subunit of CK2. Immunoprecipitates were analysed on a SDS polyacrylamide gel followed by Western blot analysis using an antibody directed against α-tubulin. The Western blot result is shown in Fig. 3A. α-Tubulin is co-immunoprecipitated with antibodies against all three subunits of CK2. This result is not unexpected since eukaryotic cells harbour tetrameric complexes composed of α'_2/β_2,α_2/β_2 and $\alpha\alpha'/\beta_2$ subunits. As shown in Fig. 1Cby using an antibody against the α-, α′- or the β-subunit we will immunoprecipitate the different isoforms of the holoenzyme. Thus, it remains possible that tubulin binds to all three subunits or only to one or two of them.

In order to study which of the subunits of CK2 might interact with tubulin directly we separated either CK2 α′ or

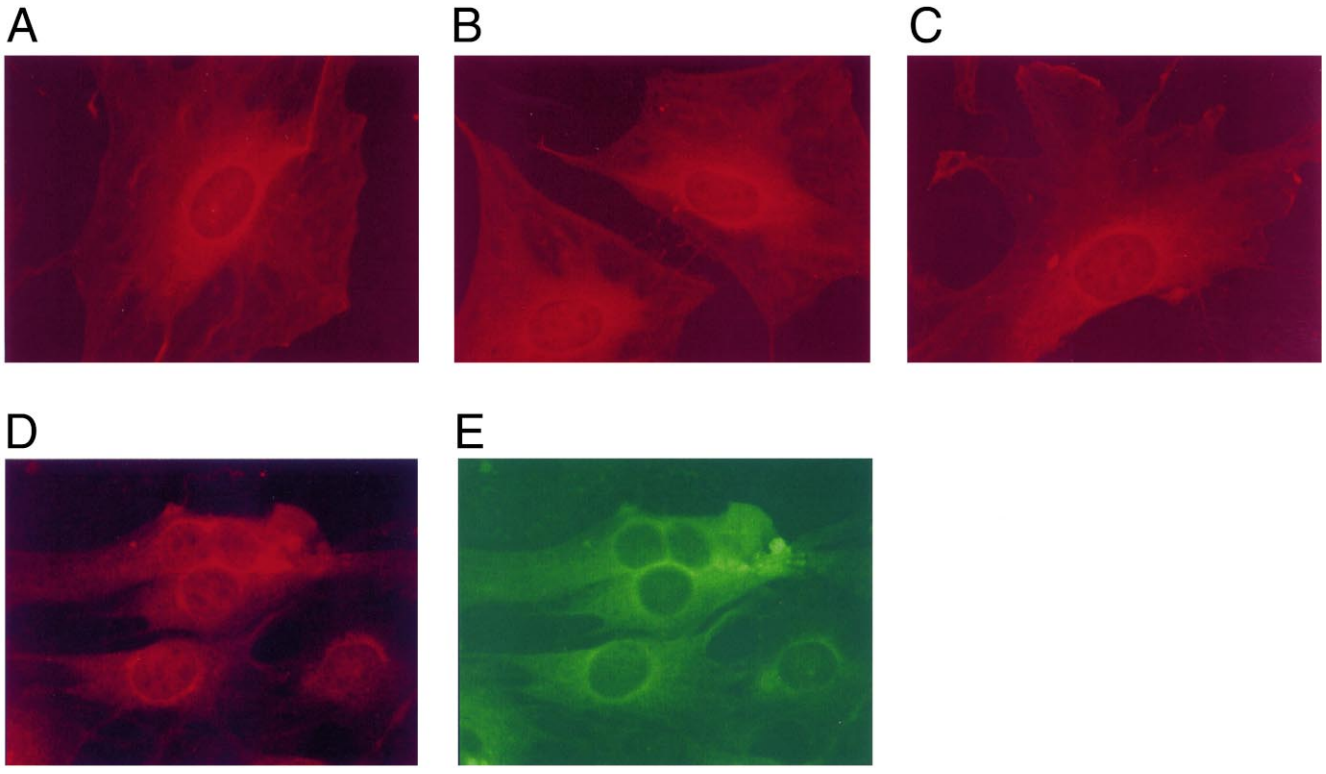

Fig. 2. Indirect immunofluorescence for the detection of individual subunits of CK2. Cells were grown on coverslips, fixed with 2% formaldehyde and permeabilised with 0.2% Triton X-100. The rabbit sera against the subunits of CK2 were used in a dilution of 1:50, the mouse monoclonal anti-α-tubulin antibody was diluted 1:1000. As secondary antibodies we used either TRITC conjugated goat anti-rabbit or FITC-conjugated goat anti-mouse-antibodies. A: serum #26, B: serum #30, C: serum #32, Double fluorescence: D: serum #26, E: anti-α-tubulin antibody.

the CK α/β holoenzyme expressed from a bicistronic vector [26] through an SDS polyacrylamide gel. After gel electrophoresis and renaturation proteins were transferred onto a PVDF membrane and the membrane was incubated with commercially available tubulin, followed by an anti-α-tubulin antibody. Binding was detected by the ECL method. As shown in Fig. 3B (lane 1), α-tubulin binds to the CK2 α′-subunit as well as to the CK2 α-subunit (lane 2). As a control, we loaded the CK2 α′ (lane 3) and the CK2 α/β holoenzyme (lane 4) on a SDS polyacrylamide gel and after transfer the proteins were detected with a mixture of serum #26, serum #30 and serum #32. These experiments demonstrated that the sample in lanes 2 and 4 contained the α and β-subunit of CK2. Since tubulin reacted only with the α- and the α′-subunit we have to conclude that there is no binding of tubulin to the β-subunit of CK2.

Next, we expressed individual subunits of CK2 in bacteria and the bacterial cell extract was incubated with tubulin. CK2 α, α′ or β were immunoprecipitated from the bacterial extract with serum #26 specific for the α-subunit, serum #30 which is specific for the α′-subunit or with serum #32 which is specific for the β-subunit. Immunoprecipitates were analysed on a SDS polyacrylamide gel followed by Western blotting with an antibody specific for α-tubulin. As shown in Fig. 3C α-tubulin was co-immunoprecipitated with the α- and the α′-subunit which is in agreement with the results shown in the Far Western analysis. There was no co-immunoprecipitation of α-tubulin with the β-subunit of CK2 which confirms the result obtained with the Western blot experiments shown in Fig. 3B.

4. Discussion

Using antibodies against the CK2 holoenzyme or the α- or β-subunit it was demonstrated that CK2 is localised either in the cytoplasm or in the nucleus [6,7,31–34]. Due to the extended homology between the α- and the α′-subunit in these early studies it was impossible to discriminate between the α- and the α′-subunit. Therefore, in the present study, we generated antibodies against peptides derived from the unique region of the α- as well as from the unique region of the α′-subunit. Antibodies against similar peptides of the individual subunits [35,36] were used to detect different isoforms of CK2 consisting of $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$ and $\alpha_2\beta_2$ in mammalian cells, which were shown to assemble with different kinetics [35]. Recently, these antibodies were used for microinjection experiments where it was shown that they interfere severely with proliferation stimulation of cells [36]. It was reported earlier that antibodies against the holoenzyme did not react with the β-subunit [31] which means that any information about the β-subunit is questionable in studies where antibodies against the holoenzyme were used. By using our newly generated antibodies we show that they immunoprecipitate the individual subunits of CK2 as well as the CK2 holoenzyme. Furthermore, it was already shown that our antibody directed against the β-subunit not only precipitated the holoenzyme but also that this immunopurified holoenzyme still has an efficient kinase activity [17]. In immunofluorescence studies these antibodies stained primary mouse embryo fibroblasts in the nucleus and in the cytoplasm with a strong accumulation around the nuclear membrane. Staining of the nucleus is

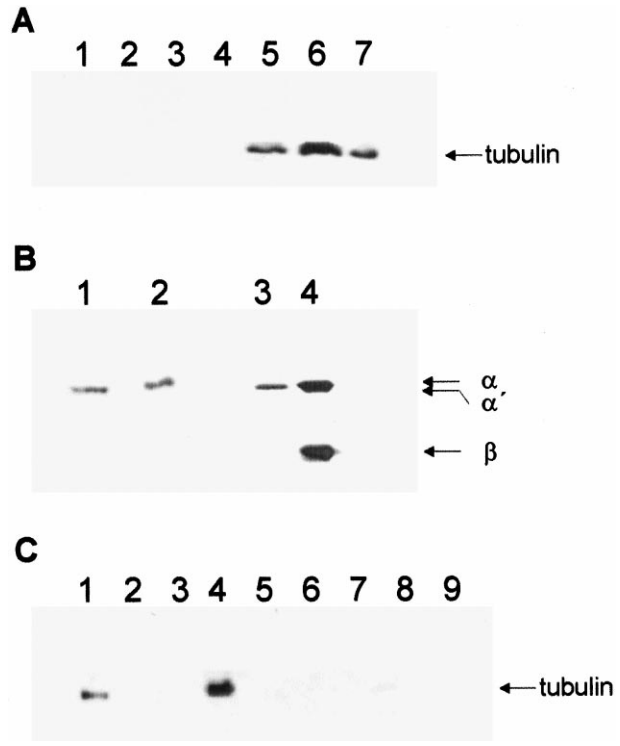

Fig. 3. Binding of CK2 α and α′ to α-tubulin. A: Co-immunoprecipitation of CK2 α and α′ with α-tubulin from mouse embryo fibroblasts. A protein A/G-sepharose mixture was preincubated with 50 μl of the sera (#26, #30 or #32) against the α-, α′- or β-subunit of CK2. One miligram cell extract of mouse embryo fibroblasts was preincubated with a mixture of protein A/G sepharose. The supernatant was applied to the preincubated sepharose-antibody-matrix. After 1 h the supernatant was removed and the matrix was washed. The immunoprecipitates were separated through an SDS polyacrylamide gel, transferred onto a PVDF-membrane, decorated with anti-α-tubulin antibody and detected by the ECL method. Antibody control (1 μl serum #26 (lane 1), #30 (lane 2), #32 (lane 3)). Lane 4: sepharose preincubated with cell extract. Immunoprecipitate with serum #26 (lane 5), #30 (lane 6), #32 (lane 7). B: Far Western blot with CK2 α-, α′- and β-subunit and tubulin. Equal amounts of CK2 α′ (lane 1) or CK2 α/β holoenzyme (lane 2) were separated through SDS polyacrylamide gels, renaturated and transferred onto a PVDF-membrane. The membrane from one of these gels was incubated with tubulin (1 μg/μl), followed by anti-α-tubulin antibody (Sigma) and then detected by the ECL system (Amersham) and the membrane from a second gel was incubated with a mixture of serum #26, #30 and #32. Proteins were visualised by the ECL system (lanes 3 and 4). C: Co-immunoprecipitation of purified CK2 α and α′ and tubulin. A protein A/G sepharose mixture was preincubated with 50 μl of serum #26, #30 or #32, respectively. The antibody-sepharose-matrix was washed and incubated with 10 μg tubulin and equal amounts of purified CK2 α-, α′- and β-subunit (lanes 1, 4 and 7). As a control the protein A/G sepharose mixture without serum was incubated with 10 μg tubulin and equal amounts of purified CK2 α-, α′- and β-subunit (lanes 2, 5 and 8). As a further control a protein A/G sepharose mixture was preincubated with 50 μl of serum #26, #30 or #32 against the α-, α′- and β-subunit of CK2, respectively, washed and incubated with 10 μg of tubulin (lanes 3, 6 and 9). After 1 h the supernatants were removed and the matrix was washed. Immunoprecipitates were separated through an SDS polyacrylamide gel, transferred onto a PVDF-membrane and decorated with anti-α-tubulin antibody and detected by the ECL method.

weak for all three antibodies. A predominant localisation of CK2 in the nucleus was found by Krek et al. [6] whereas others found CK2 mainly in the cytosol [7]. However, in these studies different antibodies against the CK2 holoenzyme or polyclonal sera against individual subunits were used and it may well be that under various cellular conditions incomplete epitope recognition by these antibodies may explain the different subcellular localisation. Moreover, the α- and the α′-subunit interact with itself, with the β-subunit and with an increasing number of cellular and viral proteins and these protein-protein interactions may also prevent epitope recognition. We generated antibodies against regions on the polypeptide chain which to our current knowledge are not implicated in protein-protein interactions. In addition different cell types such as primary skin fibroblasts, immortalised 3T3 cells and tumour cells were used for the subcellular localisation of CK2. It is possible that the subcellular localisation of CK2 may vary with the phenotype of the cells. Moreover, there is some indication that the subcellular localisation may vary with the cell cycle [31,33]. Our present results using primary mouse embryo fibroblasts are similar to the results previously obtained for the α-subunit of CK2 in mouse 3T3 cells [31]. The staining for CK2 observed in 3T3 cells as well as in the primary mouse embryo fibroblasts used in our present study resembled the staining which was reported for α-tubulin [30] and shown also in the present study using a commercially available antibody against α-tubulin. Tubulin is not only co-localised with the three subunits of CK2 but can also be co-immunoprecipitated from mammalian cells with the antibodies directed against the α-, α′- and the β-subunit. Co-immunoprecipitation experiments with individual subunits and α-tubulin as well as Western blot experiments revealed that only the α- and the α′- but not the β-subunit bind to α-tubulin.

It is known for a long time that protein kinase CK2 phosphorylates β-tubulin in vitro and in vivo [37–39]. Moreover CK2 was also found to be associated with microtubules [40]. According to our present result CK2 may be involved in cytoskeletal reorganisation not only by phosphorylation of proteins such as tubulin and myosin but also by stable association with at least tubulin. CK2 forms stable complexes with cellular and viral proteins [1,16,41,42] and these interactions can alter the activity of the kinase itself [17,43] as well as of its binding partners such as p53, PP2A and topoisomerase [21,44–46]. One can also suppose a stable binding between CK2 and microtubules that localise the kinase very closely to its cytoskeletal substrates so that they can be phosphorylated more efficiently. The cytoskeleton plays an important role in the maintenance of cell shape and polarity and also in cell mobility. There are some reports suggesting a role of CK2 in cell polarity and differentiation. The gene for the catalytic subunit of CK2 in *Schizosaccharomyces pombe* was found as a temperature sensitive recessive lethal mutation (orb5), which confers a spherical morphology to the filamentous yeast [47]. Deletion of the gene for the regulatory β-subunit of CK2 in *S. pombe* is not lethal, but shows also an abnormal rounded morphology reminiscent of orb5 [48]. Furthermore, the temperature sensitive mutation ($cka1^{ts}$) of CK2 in *Saccharomyces cerevisiae* shows after shift to the restrictive temperature a population of budded and unbudded cells with a spherical morphology [49]. Also in neuroblastoma cells, where the activity of CK2 was deleted through antisense technology, neuritogenesis and cell polarity were inhibited [50]. Thus, these studies reveal a role for CK2 in cell polarity and differentiation. Binding of CK2 to proteins such as tubulin may target CK2 to substrates which are implicated in these processes.

Acknowledgements: We thank W. Nastainczyk for peptide synthesis, P. Leder for MEF cells, Y. Shi for CK2 constructs and O.G. Issinger for monoclonal antibodies 1AD9 and 6D5. This work was supported by Grants from Deutsche Forschungsgemeinschaft Mo309/11-1, Mo309/11-3, SFB 399-B4, Alois Lauer Stiftung and by Fonds der Chemischen Industrie to M.M.

References

[1] Guerra, B. and Issinger, O.G. (1999) Electrophoresis 20, 391–408.
[2] Pinna, L.A. and Meggio, F. (1997) Prog. Cell Cycle Res. 3, 77–97.
[3] Greenwood, J.A., Scott, C.W., Spreen, C.B., Caputo, C.B. and Johnson, G.V. (1994) J. Biol. Chem. 269, 4373–4380.
[4] Allende, J.E. and Allende, C.C. (1995) FASEB J. 9, 313–323.
[5] Karki, S., Tokito, M.K. and Holzbaur, E.L.F. (1997) J. Biol. Chem. 272, 5887–5891.
[6] Krek, W., Maridor, G. and Nigg, E.A. (1992) J. Cell Biol. 116, 43–55.
[7] Yu, I.J., Spector, D.L., Bae, Y.-S. and Marshak, D.R. (1991) J. Cell. Biol. 114, 1217–1232.
[8] Tawfic, S., Davis, A.T., Faust, R.A., Gapany, M. and Ahmed, K. (1997) J. Cell. Biochem. 64, 499–504.
[9] Guo, C.H., Davis, A.T. and Ahmed, K. (1998) J. Biol. Chem. 273, 13675–13680.
[10] Li, D.X., Dobrowolska, G. and Krebs, E.G. (1996) J. Biol. Chem. 271, 15662–15668.
[11] Filhol, O., Cochet, C. and Chambaz, E.M. (1990) Biochemistry 29, 9928–9936.
[12] Meggio, F., Brunati, A.M., Donella-Deana, A. and Pinna, L.A. (1984) Eur. J. Biochem. 138, 379–385.
[13] Kübler, D., Pyerin, W., Burow, E. and Kinzel, V. (1983) Proc. Natl. Acad. Sci. USA 80, 4021–4025.
[14] Herrmann, C.P.E., Kraiss, S. and Montenarh, M. (1991) Oncogene 6, 877–884.
[15] Appel, K., Wagner, P., Boldyreff, B., Issinger, O.-G. and Montenarh, M. (1995) Oncogene 11, 1971–1978.
[16] Götz, C., Wagner, P., Issinger, O.-G. and Montenarh, M. (1996) Oncogene 13, 391–398.
[17] Schuster, N., Götz, C., Schneider, E., Prowald, A., Faust, M. and Montenarh, M. (1999) submitted.
[18] Li, D.X., Meier, U.T., Dobrowolska, G. and Krebs, E.G. (1997) J. Biol. Chem. 272, 3773–3779.
[19] Bozzetti, M.P., Massari, S., Finelli, P., Meggio, F., Pinna, L.A., Boldyreff, B., Issinger, O.-G., Palumbo, G., Ciriaco, C. and Bonaccorsi, S. (1995) Proc. Natl. Acad. Sci. USA 92, 6067–6071.
[20] Hagemann, C., Kalmes, A., Wixler, V., Wixler, L., Schuster, T. and Rapp, U.R. (1997) FEBS Lett. 403, 200–202.
[21] Hériché, J.K., Lebrin, F., Rabilloud, T., LeRoy, D., Chambaz, E.M. and Goldberg, Y. (1997) Science 276, 952–955.
[22] Lozeman, F.J., Litchfield, D.W., Piening, C., Takio, K., Walsh, K.A. and Krebs, E.G. (1990) Biochemistry 29, 8436–8447.
[23] Issinger, O.-G. (1993) Pharmacol. Ther. 59, 1–30.
[24] Deng, C.X., Zhang, P.M., Harper, J.W., Elledge, S.J. and Leder, P. (1995) Cell 82, 675–684.
[25] Brattain, M.G., Levine, A.E., Chakrabarty, S., Yeoman, L.C., Wilson, J.K. and Long, B. (1984) Cancer Metastasis Rev. 3, 177–191.
[26] Shi, Y., Brown, E.D. and Walsh, C.T. (1994) Proc. Natl. Acad. Sci. USA 91, 2767–2771.
[27] Grankowski, N., Boldyreff, B. and Issinger, O.-G. (1991) Eur. J. Biochem. 198, 25–30.
[28] Schmidt-Spaniol, I., Boldyreff, B. and Issinger, O.-G. (1992) Hybridoma 11, 53–59.
[29] Nastainczyk, W., Schmidt-Spaniol, I., Boldyreff, B. and Issinger, O.-G. (1995) Hybridoma 14, 335–339.
[30] Blose, S.H., Meltzer, D.I. and Feramisco, J.R. (1984) J. Cell. Biol. 98, 847–858.
[31] Schmidt-Spaniol, I., Grimm, B. and Issinger, O.-G. (1993) Cell. Mol. Biol. Res. 39, 761–772.
[32] Stalter, G., Siemer, S., Becht, E., Ziegler, M., Remberger, K. and Issinger, O.-G. (1994) Biochem. Biophys. Res. Commun. 202, 141–147.
[33] Lorenz, P., Pepperkok, R., Ansorge, W. and Pyerin, W. (1993) J. Biol. Chem. 268, 2733–2739.
[34] Belenguer, P., Baldin, V., Mathieu, C., Prats, H., Bensaid, M., Bouche, G. and Amalric, F. (1989) Nucleic Acids Res. 17, 6625–6636.
[35] Chester, N., Yu, I.J. and Marshak, D.R. (1995) Biol. Chem. 13, 7501–7514.
[36] Lorenz, P., Ackermann, K., Simoes-Wuest, P. and Pyerin, W. (1999) FEBS Lett. 448, 283–288.
[37] Serrano, L., Diaz-Nido, J., Wandosell, F. and Avila, J. (1987) J. Cell Biol. 105, 1731–1739.
[38] Crute, B.E. and van Buskirk, R.G. (1992) J. Neurochem. 59, 2017–2023.
[39] Kohtz, D.S. and Puszkin, S. (1989) J. Neurochem. 52, 285–295.
[40] Serrano, L., Hernandez, M.A., Diaz-Nido, J. and Avila, J. (1989) Exp. Cell Res. 181, 263–272.
[41] Götz, C., Koenig, M.G., Issinger, O.-G. and Montenarh, M. (1995) Eur. J. Biochem. 233, 327–334.
[42] Götz, C., Scholtes, P., Schuster, N., Prowald, A., Nastainczyk, W. and Montenarh, M. (1999) Mol. Cell. Biochem. 191, 111–120.
[43] Guerra, B., Götz, C., Wagner, P., Montenarh, M. and Issinger, O.-G. (1997) Oncogene 14, 2683–2688.
[44] Schuster, N., Prowald, A., Schneider, E., Scheidtmann, K.-H. and Montenarh, M. (1999) FEBS Lett. 447, 160–166.
[45] Prowald, A., Schuster, N. and Montenarh, M. (1997) FEBS Lett. 408, 99–104.
[46] Redwood, C., Davies, S.L., Wells, N.J., Fry, A.M. and Hickson, I.D. (1998) J. Biol. Chem. 273, 3635–3642.
[47] Snell, V. and Nurse, P. (1999) EMBO J. 13, 2066–2074.
[48] Roussou, I. and Draetta, G. (1994) Mol. Cell. Biol. 14, 576–586.
[49] Rethinaswamy, A., Birnbaum, M.J. and Glover, C.V.C. (1998) J. Biol. Chem. 273, 5869–5877.
[50] Ulloa, L., Diaz-Nido, J. and Avila, J. (1993) EMBO J. 12, 1633–1640.