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Research Article

Specific localization of the catalytic subunits of protein kinase CK2 at the centrosomes

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Abstract. The protein kinase CK2 holoenzyme is composed of two regulatory β subunits and two catalytic α or α' subunits. Although experimental evidence for involvement of the enzyme in the regulation of cell proliferation is accumulating, the exact mechanism of its action is still unclear. The subcellular localization of the enzyme may be a key to its function. We have recently shown that the CK2 holoenzyme is tightly associated with the Golgi complex and the endoplasmic reticulum. Centrosomes, which organize spindle formation during the cell cycle and microtubule cytoskeleton formation and, thereby, the location and orientation of different organelles in the cell, are in close vicinity to the Golgi complex. Because several kinases and phosphatases have been described to regulate the functions of the centrosome, we analysed the association of CK2 with these organelles. Using biochemical cell fractionation and coimmunoprecipitation, we never found the holoenzyme but only the catalytic α subunits associated with the centrosome. These data were confirmed by immunoelectron microscopy. Thus, the present data point to a particular role of the catalytic α and α' subunit of protein kinase CK2, which may be different from their roles in the holoenzyme.

Key words. Centrosome; casein kinase 2; catalytic subunit; immunofluorescence; subcellular localization.

About 100 years ago, Theodor Boveri described a small, central body near the nucleus of the cell, which he called the 'centrosome'. The best-known function of the centrosome is its ability to nucleate and organize microtubule growth, thus representing a microtubule organization centre. The most prominent structure organized by the centrosomes is the spindle apparatus, which is necessary for the segregation of the chromosomes during mitosis. These events have to be strictly controlled to guarantee the fidelity of this process. Duplication of the centrosome should occur exactly once during the cell cycle, so that a bipolar spindle is formed and not a monopolar one as would occur if the centrosome failed to duplicate, or a multipolar one in cases of multiple replication. Moreover, the centrosome cycle has to be coordinated with the cycle of DNA replication and cell division, and protein phosphorylation is one of the key regulating functions of the centrosome cycle [1, 2]. Vandre and Borisy [3] detected a significant increase in the phosphorylation status of centrosome proteins during mitosis. Several protein kinases could also be localized to the centrosome during mitosis or throughout the cell cycle. Among them, cyclin-dependent kinases (e.g. cdk1 and cdk2) [4, 5], polo-like kinases (e.g. Plk-1) [6], NIMA-related kinases (nek-2) [7], Aurora kinases (e.g. STK15) [8], protein kinase A (PKA) and protein kinase C (PKC) [9–11] and also phosphatases (e.g. PP1α) [7] have been identified. The exact biological role of these kinases for the duplication, matu-

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ration and segregation of the centrosomes is only known for a few of these enzymes. The significance of these enzymes becomes evident when they are deregulated, as is known for the Aurora kinase STK15 [12]. STK15 is amplified in some tumours, resulting in an elevated kinase activity. On the other hand, in these tumours, centrosome amplification was observed. It is tempting to speculate [13] that the increase in kinase activity leads to a centrosome dysfunction and to an improper segregation of the chromosomes, thus providing a prerequisite for carcinogenesis.

Protein kinase CK2 activity is also elevated in human tumour cells compared to normal non-transformed cells [14, 15]. This enzyme phosphorylates a variety of different classes of proteins in the cell including cytoskeleton proteins, transcription factors and signal transduction proteins [16]. Protein kinase CK2 is a ubiquitous Ser/Thr protein kinase with a preference for acidic sequences, which has been found at different locations within the cell [for a review see ref. 17]. Active CK2 is a heterotetrameric enzyme consisting of two catalytic subunits (α and/or α') and two regulatory β subunits [18]. Most of the known substrates are phosphorylated by the holoenzyme, but recently, several functions different from those of the holoenzyme have been attributed to the individual subunits [19–21]. Moreover, a recent report shows that the activity of the α subunit alone is differently regulated than the α subunit in complex with the β subunit [22], providing strong support for the hypothesis that both forms fulfil different tasks in the cell. The exact function of the kinase is not yet known. It participates in a multitude of essential pathways within the cell and, obviously, life is not possible without this enzyme [23, 24].

We recently described the localization of protein kinase CK2 at the endoplasmic reticulum (ER) and the Golgi complex, which are located close to the centrosome [25]. Moreover, all these structures maintain a functional relationship, because microtubules organized by the centrosomes determine the exact location of the ER and Golgi complex within the cell. We detected the catalytic α and regulatory β subunits at the smooth ER and at the Golgi complex, whereas the rough ER only harboured the catalytic α and α' subunits, indicating individual roles for the catalytic subunits at specific places in the cell. As the Golgi complex lies close to the centrosome in mammalian cells and cannot be sufficiently differentiated by immunofluorescence techniques, we decided to isolate the centrosomes by biochemical techniques and look for the distribution of the individual CK2 subunits in these organelles. By fractionation, coimmunoprecipitation and immunoelectron microscopy experiments, we found that only the catalytic α and α' subunits of CK2 associate with the centrosomes. Thus, like the rough ER, the centrosomes only harbour the catalytic subunits of protein kinase CK2. Therefore, the present study together with the previous one [25] strongly supports the notion of a special function of the catalytic subunits of CK2, differing from their role in the holoenzyme.

Materials and methods

Cells

cos-1 cells are SV40-transformed monkey kidney cells [26], Saos-2 are human osteosarcoma cells [27]. Both lines were maintained in Dulbecco´s modified Eagle´s medium (DMEM) supplemented with 10% foetal calf serum (FCS). Cells were grown to 75% confluence in 10-cm dishes in a humidified 5% CO_2 atmosphere. For the preparation of cell extracts, cells were harvested with a rubber policeman and washed three times with phosphate-buffered saline (PBS), pH 7.4. The cell pellet was lysed with a double volume of lysis buffer (100 mM Tris-HCl, pH 7.0, 100 mM NaCl, 0.5% NP40, 1% Trasylol). Proteins were extracted for 1 h on ice. Cell debris was eliminated by centrifugation (4 °C, 30 min, 13,000 g).

Antibodies

For the detection of CK2 in Western blot, immunofluorescence, immunoprecipitation and immunoelectron microscopy, we used rabbit serum No. 26 for CK2 α, serum No. 30 for CK2 α' and serum No. 32 for CK2 β. These sera were raised against amino acids 360–371 of CK2 α, amino acids 330–349 of CK2 α' and amino acids 206–215 of CK2 β and have already been described [28]. Antibodies against α- and γ-tubulin are commercially available from Sigma-Aldrich (Deisenhofen, Germany).

Immunofluorescence

Cells were grown on coverslips to 50–70% confluence, then fixed in 2% formaldehyde in PBS, pH 7.4, for 15 min at 20 °C and finally washed with PBS, pH 7.4, for 3 × 10 min. Cells were permeabilized 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 three times for 10 min and then incubated with a primary antibody at 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 (FITC or TRITC conjugated; Alexafluor, Molecular Probes, Leiden, The Netherlands) at room temperature for 1 h in a dark, humidified chamber. Finally, cells were fixed with a drop of mounting medium and analysed with a fluorescence microscope.

Immunoprecipitation

For immunoprecipitation, we used serum No. 26 and a monoclonal anti-γ-tubulin antibody (Sigma). A protein A/G-sepharose mixture was pre-incubated for 1 h with

50 µl of the serum or with 10 µl (1 µg/µl) of the different monoclonal antibodies and washed three times with PBS, pH 7.4. One milligram of cell extract was pre-incubated with a mixture of protein A- and protein G-sepharose (Amersham-Pharmacia, Freiburg, Germany) to remove unspecific-binding proteins. The supernatant was applied to the pre-incubated sepharose-antibody matrix and incubated for 1 h. The supernatant was removed and the antibody matrix was washed five times with PBS. The immune complex was subjected to SDS-polyacrylamide gel electrophoresis followed by Western blotting.

Immunoelectron microscopy

For immunolabelling, cells were fixed with 4% paraformaldehyde and 0.1% glutaraldehyde at 4 °C, dehydrated with ethanol while progressively lowering the temperature and embedded at –30 °C in Lowicryl HM20 (Chemische Werke Lowi, Waldkraiburg, Germany). Ultra-thin sections were labelled with rabbit anti-CK2 α (serum No. 26), -CK2 α' (serum No. 30) or -CK2 β antibodies (serum No. 32) [28]. After washing, a secondary goat anti-rabbit 10-nm gold antibody (Aurion, Wageningen, The Netherlands) was used. Serial sections were stained with uranyl acetate and lead citrate and examined with an EM109 transmission electron microscope (Carl Zeiss, Oberkochen, Germany).

Computer-assisted three-dimensional reconstruction

To gain a better insight into the spatial relationship between centrioles and CK2 labels, we used a modelling technique from serial ultra-thin sections (BioModel software) described elsewhere [29]. Cross-sectioned centrioles labelled with antibodies against the α and α' subunits of CK2 were selected for these reconstructions. The structural elements used for reconstruction (centriolar microtubules) and the gold particles bound to them were digitized from the micrographs using a Wacom input system.

Isolation of centrosomes

All solutions were prepared shortly before use and kept at 4 °C. cos-1 cells were usually seeded onto 150-mm plates in DMEM + 10% FCS 2 days prior to use. Best results were obtained when cells were confluent on the day of preparation. The first step of the isolation procedure was the depolymerization of cytoskeleton elements to release the centrosomes from nuclei and cytosol. Therefore, we applied nocodazole and cytochalasin B (final concentration 20 µg/ml each) for 90–120 min at 37 °C in a CO_2 atmosphere to depolymerize the tubulin and actin cytoskeleton. Then, cells were washed consecutively with PBS, tenfold-diluted PBS with 8% sucrose (w/v) and finally with 8% sucrose in H_2O. Low-ionic lysis buffer [1 mM Tris-HCl, pH 8.0, 0.1% 2-mercaptoethanol, 0.5% Triton X-100, protease inhibitor cocktail Complete (Roche Diagnostics, Mannheim, Germany)] was added and dishes were rocked gently for 10 min at 4 °C. Lysates were collected and centrifuged for 5 min at 1500 g to pellet residual nuclei and other cellular debris. The cleared lysates were poured through a filter (No. 3180, 29–33 µm; Schleicher und Schüll, Dassel, Germany), which also retained residual chromatin and cellular debris. Then, the lysates were transferred to centrifuge tubes on top of 1–1.5 ml 20% (w/v) Ficoll (MW 400,000) in PE buffer (10 mM PIPES, pH 7.2, 1 mM EDTA, 0.1% 2-mercaptoethanol) with 0.1% Triton X-100. Tubes were centrifuged at 25,000 g in a swing-out rotor for 20 min at 4 °C. The centrosome fraction was found 2 ml above the Ficoll cushion and was collected with a glass Pasteur capillary pipette. The fractions were diluted 1:4 in PE buffer and centrifuged at 25,000 g for 20 min at 4 °C. The sediment was resuspended in sample buffer (130 mM Tris-HCl, pH 6.8, 0.02% bromophenol blue, 5 mM EDTA, 10% 2-mercaptoethanol, 20% glycerol) and loaded onto an SDS-polyacrylamide gel.

SDS-polyacrylamide gel electrophoresis and Western blot

Proteins were analysed by SDS-polyacrylamide gel electrophoresis according to the procedure of Laemmli [30]. For Western blot analysis, proteins were transferred to a PVDF membrane by tank blotting with 20 mM Tris-HCl, pH 8.7, and 150 mM glycine as transfer buffer. 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 antibody (usually at a dilution of 1:1000, unless otherwise stated) in PBS-Tween 20 with 1% dry milk for another hour. The membrane was then washed with PBS-Tween 20 three times before incubating with the peroxidase-coupled secondary antibody at a dilution of 1:30,000 in PBS-Tween 20 with 1% dry milk. Signals were developed and visualized by the Lumilight system of Roche Diagnostics.

Results

Protein kinase CK2 is colocalized with tubulin

We recently found the α and the α' subunit of CK2 associated with the rough ER whereas the holoenzyme consisting of α or α' and β subunits was associated with the smooth ER and the Golgi apparatus [25]. Since we also found CK2 α binding to tubulin [28], we now asked whether and where we might find a colocalization of CK2 and tubulin in living cells. For these studies we used rabbit sera which were generated against chosen epitopes in the C terminus of the individual subunits. These sera (No. 26 for the α subunit, No. 30 for the α' subunit, and No. 32 for the β subunit) have already been characterized in Western blot analysis and immunoprecipitation [28]. Fig-

ure 1a demonstrates their specificity in immunofluorescence by competition studies with increasing concentrations of the respective peptide. Cells grown on coverslips were either labelled with serum No. 26 (A), serum No. 30 (B) or with serum No. 32 (C) and a secondary TRITC-conjugated antibody. Panel 1 shows the immunostaining in the absence of a neutralizing peptide, panels 2 and 3 in the presence of the respective neutralizing peptides at increasing concentrations (10 and 100 µM, respectively). In every case, immunostaining disappeared in the presence of the antigens. The specificity of the antibodies was further tested in Western blot experiments using a total cell extract from HeLa cells. A protein extract from HeLa cells was separated in several lanes by SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane. Lanes were blotted either with serum No. 26 (lane 1), serum No. 30 (lane 2) or serum No. 32 (lane 3). After developing the signals by enhanced chemiluminescence, no other signals besides those for the corresponding CK2 subunits could be visualised in a total cell extract, even after prolonged incubation (fig. 1b). Thus, we were sure that the antibodies recognized only their specific antigen and we used these sera for colocalization studies in cos-1 cells, which are characterized by large centrosomes. Cells were grown on coverslips, fixed and labelled with rabbit antibodies against the individual subunits of CK2 and a mouse anti-α-tubulin antibody. The secondary antibodies against the rabbit sera were conjugated with TRITC; those against the mouse monoclonal antibody were conjugated with FITC. The nuclei of the cells were counterstained with DAPI. The results obtained with the anti-CK2 α serum are shown in figure 2. The results obtained with the anti-CK2 α' and the anti-CK2 β serum were quite similar (not shown). We observed a predominantly nuclear and to a lesser extent a cytoplasmic staining for the CK2 α subunit (red fluorescence). In addition, cytoskeleton fibres as well as centrosome structures were labelled with these antibodies. Cytoskeleton as well as centrosome structures were also identified with the anti-α-tubulin antibody (green fluorescence). By merging the red and the green fluorescence, we found regions where both antibodies colocal-

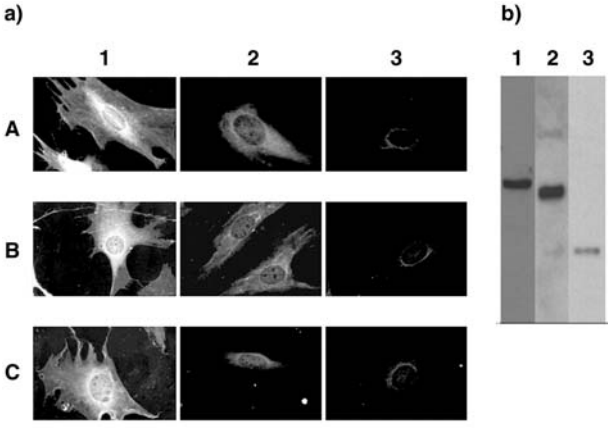

Figure 1. Specificity of CK2 antisera. (*a*) Saos-2 cells were grown on coverslips and either labelled using serum No. 26 for CK2 α (A), serum No. 30 for CK2 α' (B) or serum No. 32 for CK2 β (C). The immunostaining was performed in the absence (1) or presence of increasing concentrations (2 and 3: 10 and 100 µM, respectively) of the respective peptides (corresponding to amino acids 360–371 for CK2 α, amino acids 330–349 for CK2 α' and amino acids 206–215 for CK2 β.) The primary antibodies were detected with a secondary TRITC-conjugated secondary antibody. Magnification is × 400. (*b*) Total extracts from HeLa cells were subjected to a Western blot analysis with either serum No. 26 (1), serum No. 30 (2) or serum No. 32 (3). Signals were developed in a peroxidase-dependent reaction generating chemiluminescence.

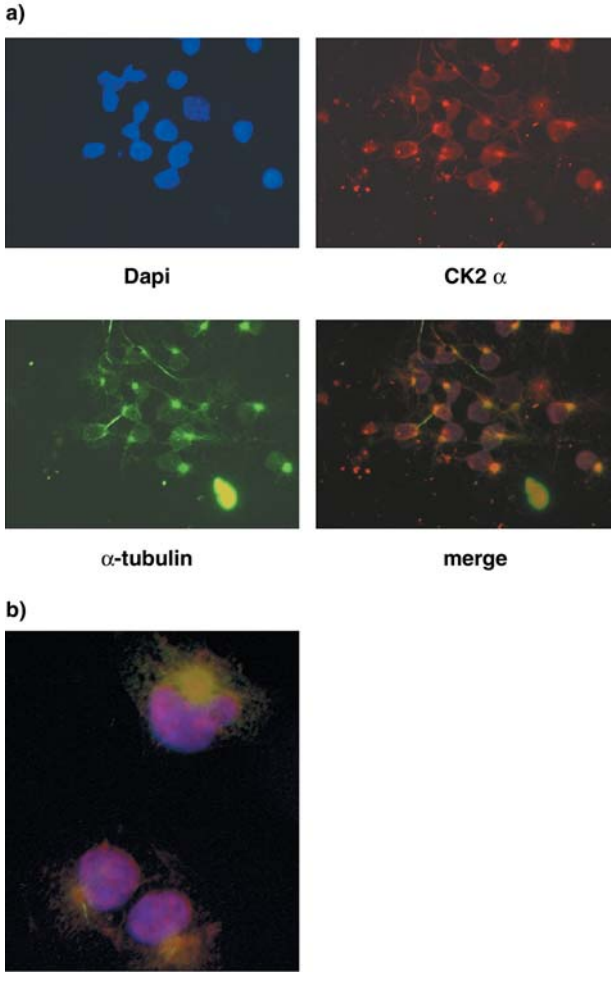

Figure 2. Co-localization of the catalytic α subunit of CK2 with tubulin. (*a*) Subconfluent growing cos-1 cells fixed with 2% formaldehyde and permeabilized with 0.2% Triton-X 100 were analysed with rabbit serum against the CK2 α subunit in a dilution of 1:100. Staining was carried out with mouse monoclonal antibodies against α-tubulin. TRITC- and FITC-conjugated anti-mouse/rabbit antibodies were used as secondary antibodies. Nuclear staining was performed with 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI; Roche Diagnostics) according to the manufacturer's instructions. (*b*) Merging of the triple staining shown in more detail.

ized, which resulted in a yellow fluorescence. These common regions were concentrated in an area close to the nucleus, which could be centrosomes or Golgi complex. Figure 2b shows the structures at a higher magnification.

Only the catalytic α subunits of protein kinase CK2 are associated with centrosomes

To determine if, besides the already described association with the Golgi complex, CK2 is also localized to the centrosomes, we attempted to enrich the centrosomes by biochemical techniques. We started by isolating the organelles according to the protocol of Blomberg-Wirschell and Doxsey [31] that results in functional centrosomes with nucleating activity. The cytoskeleton of cos-1 cells was destroyed by the application of nocodazole and cytochalasin B. Figure 3 frame 1 shows the cells stained with α-tubulin-specific antibodies before the treatment. Figure 3 frame 2 shows the anti-α-tubulin-reactive parts of the cells after the destruction of the cytoskeleton. After lysis of the cells, only the nuclei (stained with DAPI in frame 3 of fig. 3) remained on the dish; the anti-α-tubulin-reactive material was found in the lysate (compare frames 4 and 5 of fig. 3). To remove nuclei and other high-molecular-weight compounds, the lysates were subjected to a Ficoll density gradient centrifugation. The presence of centrosomes in the purified fraction was verified by labelling with a γ-tubulin-specific antibody (frame 6 of fig. 3). To study the association of CK2 with centrosomal structures, we analysed the purified centrosomal extracts from the Ficoll centrifugation in a Western blot analysis with CK2-specific antibodies. Figure 4 shows the results of an immunoblot with total extracts from cos-1 cells and of the centrosomes purified by Ficoll density gradient centrifugation. The extracts were stained with antibodies specific for CK2 α (serum No. 26), CK2 α' (serum No. 30) and CK2 β (serum No. 32). All three subunits could be well detected in the total cell extract of cos-1 cells. In the extract of the centrosomes, we only detected the catalytic α subunit and the α' subunit. The CK2 holoenzyme was shown to be a very stable enzyme that only dissociates after treatment with denaturing agents [32]. The procedure for the isolation of centrosomes was chosen because it has been described as suitable for localizing centrosome proteins by immunofluorescence and biochemical techniques. Thus, the CK2 β subunit is unlikely to have got lost during the preparation. Since we were unable to detect the regulatory β subunit in any of the fractions, we suggest that in the centrosome compartment of the cell, CK2 does not exist as a heterotetrameric holoenzyme.

In the above-described experiments we showed that the CK2 α and α' subunits are colocalized with centrosome marker proteins in cos-1 cells and we further demonstrated by biochemical techniques that only the α and α'

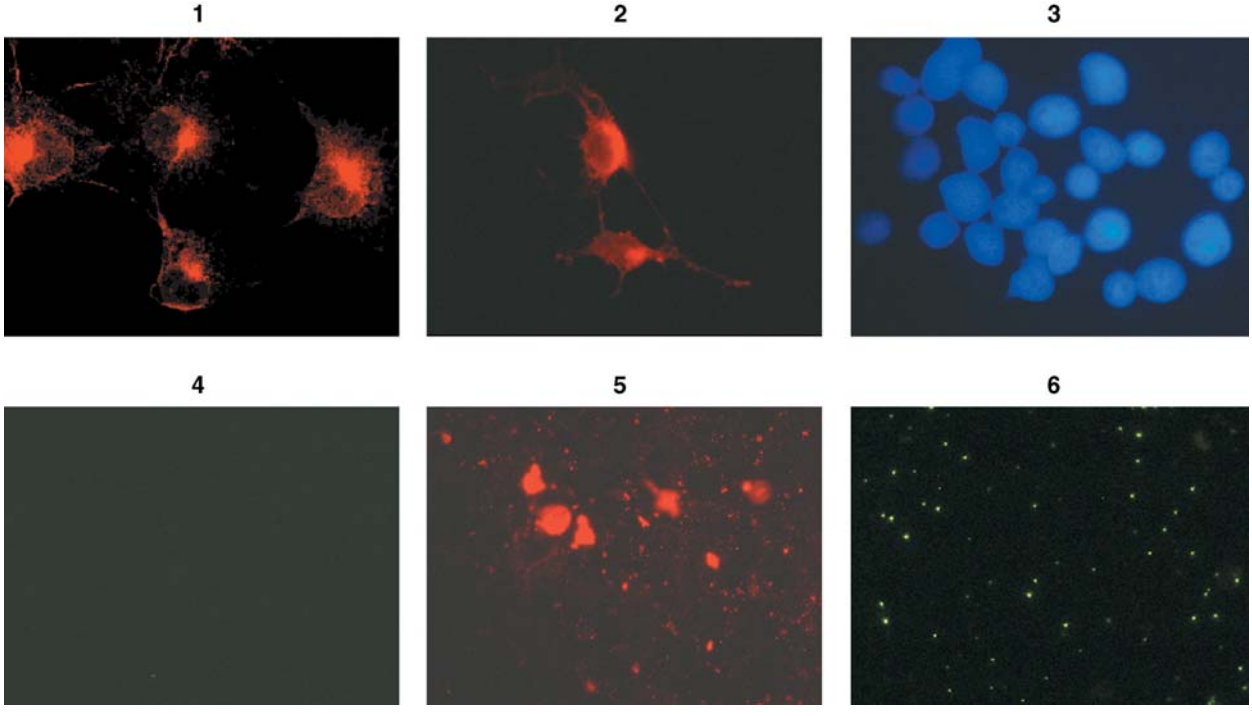

Figure 3. Purification of centrosomes. 1, cells before nocodazole and cytochalasin B treatment, immunostained with anti-α-tubulin antibodies; 2, cells after nocodazole and cytochalasin B treatment stained with anti-α-tubulin antibodies; 3, nuclei after lysis remaining on the petri dish, stained with DAPI; 4, same as 3 but stained instead with anti-α-tubulin antibodies; 5, lysate before filtration; 6, centrosomes after Ficoll-purification, stained with anti-γ-tubulin antibodies.

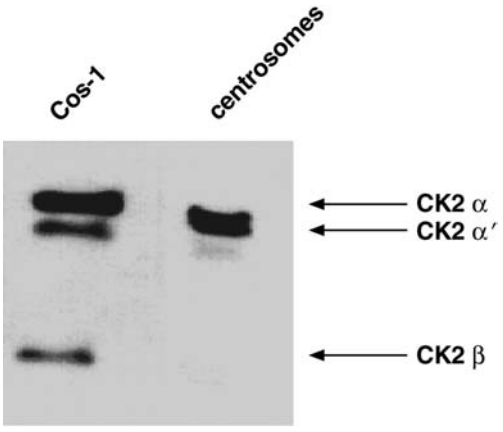

Figure 4. Detection of protein kinase CK2 in centrosome extracts. cos-1 cells were plated on 150-mm plates in DMEM + 10% FCS 2 days prior to use. Cells were treated with nocodazole and cytochalasin B (final concentration of 20 µg/ml) to depolymerize the tubulin and actin cytoskeleton. After washing with different buffers, cells were lysed. The lysate containing the centrosomes was centrifuged and the pellet was resuspended and loaded on an SDS-polyacrylamide gel, transferred onto a PVDF membrane, decorated with sera No. 26, 30 and 32 and developed by the ECL method. A cos-1 cell extract was used as a control; proteins were detected with the same antibody mix.

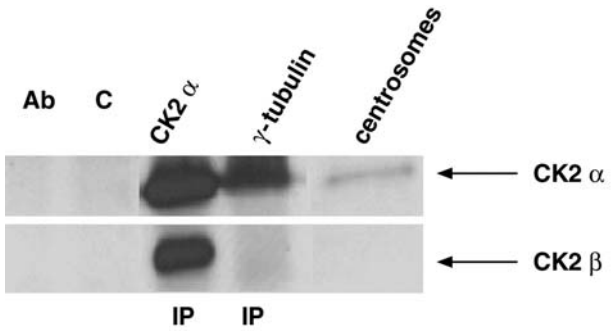

Figure 5. Coimmunoprecipitation of CK2 with γ-tubulin from cos-1 cells. A protein A/G-sepharose mixture was pre-incubated with 50 µl of the serum (No. 26) against the α subunit of CK2 or with 10 µl of a monoclonal anti-γ-tubulin antibody and then incubated with a total cell extract from cos-1 cells. The immunoprecipitates were separated through an SDS-polyacrylamide gel, transferred onto a PVDF membrane, decorated with sera No. 26 and 32 and visualized by the ECL method. Antibody control (Ab), sepharose pre-incubated with cell extract (C), immunoprecipitate with serum No. 26 (IP CK2 α), anti-γ-tubulin (IP γ-tubulin), preparation of centrosomes (centrosomes).

subunits appeared in the same fractions as the centrosome protein γ-tubulin. To investigate whether CK2 is tightly associated with the centrosomes, we used the immunoprecipitation technique. Total extracts of cos-1 cells were either incubated with the CK2 α-specific antibody No. 26 as positive control or with a γ-tubulin-specific antibody. Immunocomplexed proteins were eluted and analysed by SDS-polyacrylamide gel electrophoresis and Western blot analysis with CK2 α- and CK2 β-specific antibodies (fig. 5). In immunoprecipitates with serum No. 26 (lane CK2α), we detected both CK2 subunits. In contrast, in immunoprecipitates with the γ-tubulin-specific antibody (lane γ-tubulin), only the catalytic α subunit could be identified by the Western blot analysis as a coimmunoprecipitated protein.

Thus, the immunofluorescence analysis as well as biochemical techniques showed that the catalytic subunits are localized at the centrosome. To further support these results, we employed immunoelectron microscopy, which should allow us to determine the exact localization at a subcellular level. We again used the above-described sera, which were shown to be very specific for the individual subunits. Figure 6a–h shows the labelled sections of cos-1 cells. We focused on the centrosomes with their characteristic structure and only show these results; the antibodies, as expected, stained all other known compartments like nuclei, rough ER and exocytotic organelles. The core of the centrosome is a pair of centrioles. Each centriole is a hollow cylinder, composed of nine triplets of specialized microtubules forming the wall of the centriole. This structure was clearly visible in cross-sectioned centrioles (fig. 6a, b). Longitudinally sectioned centrioles looked barrel-shaped (fig. 6c, d). Gold labels at the centrioles could only be detected on the centrioles when labelled with CK2 α (fig. 6a, b) or CK2 α′ antibodies (fig. 6c–f). Gold labels were absent on the centrioles when the labelling was carried out with the CK2 β antibody (fig. 6g, h). On the other hand, the antibody detected CK2 β on the ER in vesicles (fig. 6g) or within the nucleus (not shown). All antibodies used in the present study recognize only one epitope on the polypeptide chain and, therefore, it is not surprising that we obtained only a few labels for the individual subunits. However, the electron microscopy results supported the results of the biochemical analysis at the ultrastructural level. A three-dimensional reconstruction from eight serial sections of centrioles labelled with antibodies against the α and α′ subunits of CK2 shows the label density on the whole centriole (figs. 7a, b). Thus, by using high-resolution immunoelectron microscopy, we support the results of the biochemical cell fractionation experiments.

Discussion

Protein kinase CK2 is a ubiquitous, pleiotropic Ser/Thr protein kinase, which was discovered in 1954 by Burnett and Kennedy [33]. Because of its ubiquitous presence and the extreme abundance of substrates (about 200) [16], assigning a function to this enzyme was difficult. Protein kinase CK2 is found at various places within the cell. In addition to its location as an ectokinase on the plasma membrane and within the lumina of organelles, CK2 was found in the cytoplasm, at the cytoskeleton and

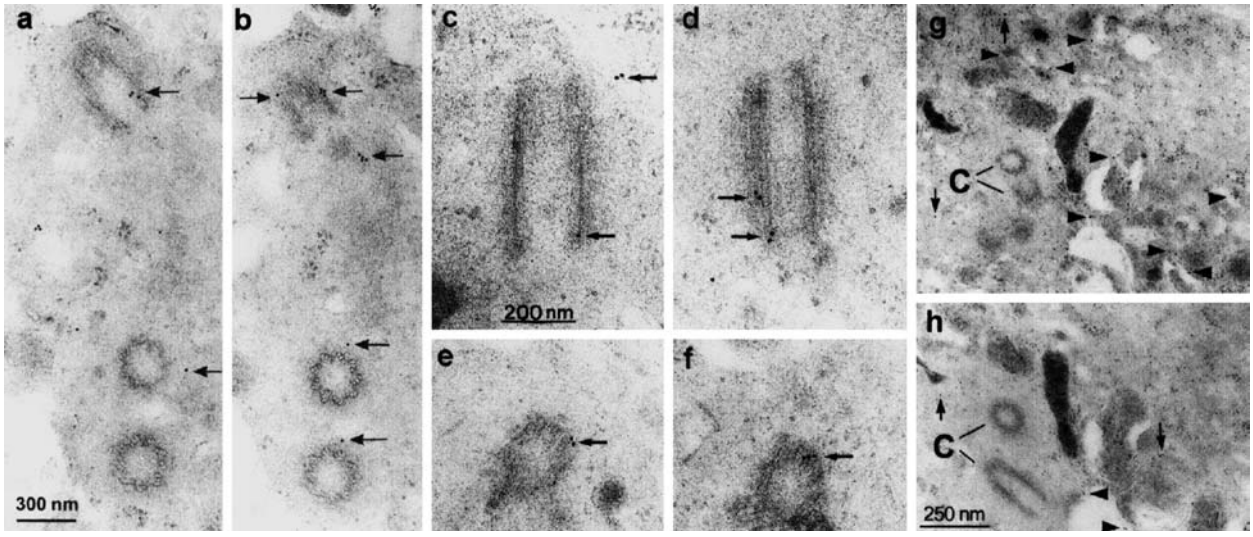

Figure 6. Immunoelectron microscopy of cos-1 cells. In panels *a* and *b,* three centrioles are seen in two sections of a series of eight sections. The centrosome at the top is obliquely sectioned; the two centrioles below are cross-sectioned. Gold labels for the CK2 α subunits are indicated with arrows. In panels *c* and *d*, two longitudinally sectioned centrioles are seen in two sections of a series of eight. In panels *e* and *f*, a centriole obliquely sectioned at its end is seen in two sections of a series of eight. Gold labels for the CK2 α subunits are indicated with arrows. In panels *g* and *h*, gold labels for the CK2 β subunit are shown in sections 1 and 3 of a series. The centriole (indicated with C) is free of labels. The CK2 β subunit was located in the vicinity of the ribosomes within the cytosol as well as on the ER (arrows) or in vesicles (arrowheads).

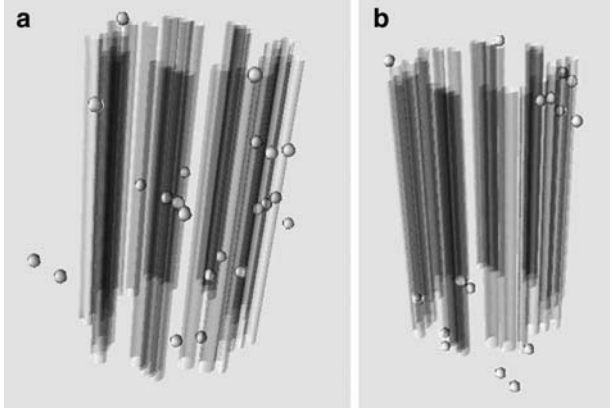

Figure 7. Three-dimensional reconstructions. To visualize the label density of α and α' subunits on the centrioles, we used micrographs from eight consecutive sections to reconstruct a schematic spatial representation of the structural features of the centrioles. It illustrates the location of both CK2 α and α' subunits on the centrioles, and especially their clustering. In this scheme, the centrioles are depicted as a translucent microtubular architecture and the opaque gold particles are attached to the organelles. (*a*) Labels for the CK2 α subunit, (*b*) Labels for the CK2 α' subunit.

the mitotic spindle and, predominantly, in the nucleus [17]. Therefore, the need was urgent to study in more detail CK2 at its different locations and, in a further step, to identify its function at specific locations. This approach might help to elucidate its precise role(s) in the cell. In a previous study, we observed that the catalytic α and α' subunits but not the regulatory β subunits associate tightly with tubulin, a component of microtubules, in primary mouse embryo fibroblasts [28]. In the present study, we found that cos-1 cells showed a strong staining for CK2 at the cytoskeleton and, moreover, most probably at the centrosomes. In an other study, we found CK2 at the smooth and rough ER, with the smooth ER and the Golgi complex harbouring all three subunits whereas the rough ER only harboured the catalytic α subunits [25]. As the Golgi complex is located near the centrosome in the cell, a clear attribution of CK2 to the centrosomes might be difficult. By coimmunofluorescence studies as well as by biochemical separation of centrosomes followed by gel electrophoresis and Western blot analysis, we always found an association of the catalytic α and α' subunits at the centrosome whereas we never detected an association of the β subunits with centrosomes. This observation is in agreement with the results of McKendrick et al. [34] who also found the catalytic subunits of CK2 associated with the centrosomes. In that study, the association of the β subunit with the centrosomes was not analysed. These and our results are in contrast to the immunofluorescence analysis of Krek et al. [35] who found only the regulatory β subunits of CK2 associated with chicken centrosomes. As this observation was obtained by immunofluorescence analysis, the observed differences might be explained by limitations of the two-dimensionality of the immunofluorescence technique. Another explanation for the different results may be that antibodies against different epitopes of CK2 β were used. Krek et al. [35] used a polyclonal serum against the β subunit, whereas we used a serum

against a peptide derived from the C terminus of the regulatory subunit, which has been thoroughly tested before in all applications, especially with respect to possible cross-reactions. Alternatively, the localization of CK2 within cells may vary depending on the cellular system.

From the very beginning, CK2 was found in most cases as a heterotetrameric enzyme consisting of two catalytic α or α' subunits and two regulatory β subunits [18]. The heterotetramer is only dissociated with denaturing agents in vitro. Most of its substrates are only phosphorylated by the holoenzyme, but in contrast to other protein kinases, the catalytic α subunit alone can phosphorylate some substrates like calmodulin or mdm2 [36, 37]. Moreover, in the last decade, many more interaction partners for the individual subunits have been described, such as PP2A [19], nucleolin [38], topoisomerase II [39] and other nuclear components [40] for the α subunit, and NOPP140 [41], c-mos [42] and A-raf [43, 44] for the β subunit. From the present study as well as a previous one [25], we have to accept that the holoenzyme is not the only form of CK2 in the cell, but that there are situations and locations in the cell where the individual subunits act on their own. In the context of our present finding, a very interesting observation was published recently [22]. Using deletion and point mutants of CK2 α, regulation of the CK2 α subunit alone was shown to differ from that of the CK2 α subunit within the holoenzyme. Bringing these two observations together, one might speculate that the regulation of CK2 at the rough ER and at the centrosomes is different from the regulation of CK2 at the Golgi complex and in the nucleus.

The exact function of protein kinase CK2 is still enigmatic. However, this kinase is known to be involved in the regulation of the G_2/M transition not only in yeast [45] but also in mammalian cells. When applying the CK2-specific inhibitor apigenin, MCF-7 cells are arrested at the G_2/M transition [46]. Sayed et al. [47] reported that CK2 is involved in G_2 arrest and apoptosis following spindle damage in epithelial cells. This process seems to be dependent on the stepwise activation of p38 MAP kinase and CK2. Depletion or inhibition of the catalytic α subunit of CK2 in the presence of microtubule inhibitors resulted in an abrogation of the G_2 arrest. When acting at the spindle checkpoint, CK2 likely has also to control the functions of the microtubule-organizing centre, the centrosome. Centrosomes together with the cytoskeleton are dynamic structures which change their localization and their form with the different cell cycle phases [48]. The centrosome cycle seems to be strictly controlled by the action of several kinases and phosphatases, but the function of only a few is known exactly [1]. Critical regulators of the centrosome cohesion might be the NEK kinase together with PP1 phosphatase [7]. Recent papers point to a role of cdk2/cyclin E kinase [49] in concert with Mps1p-like kinase [50] and perhaps $p34^{cdc2}$/cyclin B kinase [2]. Whereas Mpsp1 kinase is responsible for the reproduction of the centrosomes by phosphorylating a still unknown substrate, both cyclin-dependent kinases phoshorylate nucleophosmin, cdk2 causing its release and the splitting of the centrosomes, cdc2 leading to the re-location of nucleophosmin to the spindle poles. Nucleophosmin is a well-known substrate of protein kinase CK2 [51] and $p34^{cdc2}$ and CK2 phosphorylate each other and enhance each other's activity [52]. Thus, perhaps CK2 supports the activity of these other kinases in a positive fashion. CK2 is unlikely to be attached to the centrosomes during the entire cell cycle, and probably undergoes dynamic changes. The shuttling and the anchoring of the catalytic subunits could be done by adaptor proteins like CKIP [53] or AKAP, as is known for PKA [11]. The localization of CK2 within the cell is highly variable [17] and the shuttling between cytosol and nuclear matrix in response to growth signals has been well documented by the group of K. Ahmed [15]. To find the signal which triggers the translocalization of CK2 to the centrosomes remains an attractive goal of this project. There is some indication for an increase in the number of centrosomes upon overexpression of CK2 in mammalian cells [M. Faust, J. Günther and M. Montenarh, unpublished data]. This might indicate that CK2 is involved in the regulation of the duplication and/or segregation of the centrosomes. Similar centrosome abnormalities were seen for the amplification of the Aurora kinase STK15 [12]. Subsequently, these deregulations lead to aneuploidy and transformation of the cells. In many tumours, multiple centrosomes per cell were found [13, 54–56] which represent marker events or even inducers of carcinogenesis. Of interest is that CK2 expression and activity are elevated in a variety of different tumours [57–62]. Moreover, CK2 α has also been suggested to be an oncogene [63] and CK2 seems to be implicated in cell transformation and tumorigenesis by phosphorylation of various proteins. However, considering the results presented here, one might speculate that specifically CK2 α at the centrosomes might contribute to oncogenesis.

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