Cell Tissue Res (2000) 301:329–340
DOI 10.1007/s004410000256

REVIEW

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Subcellular localization of protein kinase CK2

A key to its function?

Received: 10 March 2000 / Accepted: 14 June 2000 / Published online: 18 July 2000

Abstract More than 46 years ago, Burnett and Kennedy first described protein kinase CK2 (formerly known as casein kinase 2) in liver extracts. Since then, protein kinase CK2 has been investigated in many organisms from yeast to man. It is now well established that protein kinase CK2 is a pleiotropic and ubiquitous serine or threonine kinase, which is highly conserved during evolution. A great number of studies deal with substrates of CK2, but the fact that over 160 substrates exist is more confusing than elucidatory. The holoenzyme is composed of two regulatory β-subunits and two catalytic α- or α´-subunits. There is now increasing evidence for individual functions of the subunits that are different from their functions in the holoenzyme. Furthermore, more and more studies describe interacting partners of the kinase that may be decisive in the regulation of this enzyme. A big step forward has been the determination of the crystal structure of the two subunits of protein kinase CK2. Now the interactions of the catalytic subunit of CK2 with ATP as well as GTP and the interaction between the regulatory subunits can be explained. However, cellular functions of protein kinase CK2 still remain unclear. In the present review we will focus our interest on the subcellular localization of protein kinase CK2. Protein kinase CK2 is found in many organisms and tissues and nearly every subcellular compartment. There is ample evidence that protein kinase CK2 has different functions in these compartments and that the subcellular localization of protein kinase CK2 is tightly regulated. Therefore studying the subcellular localization of protein kinase CK2 may be a key to its function.

This work was supported by a grant from DFG Mo 309/11–3.

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Introduction

Protein kinase CK2 is a ubiquitously expressed pleiotropic and probably constitutively active serine or threonine kinase that can use ATP as well as GTP as cosubstrate. In many organisms, protein kinase CK2 is a heterotetramer composed of two catalytic α- or α´-subunits and two regulatory β-subunits (Fig. 1). CK2 β seems to play at least a trifunctional role: it confers stability to the holoenzyme (Meggio et al. 1992), it increases enzyme activity (Grankowski et al. 1991), and it determines substrate specificity (Bidwai et al. 1992; Meggio et al. 1992). The catalytic α-subunit is regulated not only by the β-subunit but also by polyanions; very little is known about the α´-subunit. Protein kinase CK2 was first de-

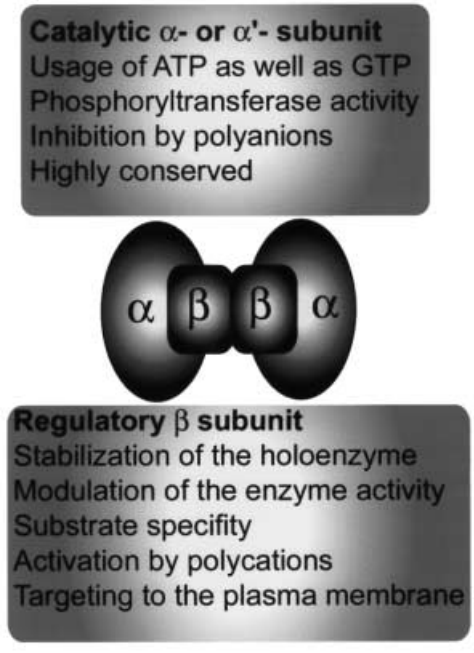


Fig. 1 Structure of protein kinase CK2 and the functions of the individual subunits

scribed in 1954 by Burnett and Kennedy in liver extracts. Since then protein kinase CK2 has been discovered in many organisms from yeast to man, indicating that CK2 has been present in living organisms for a billion years, presumably formed at the time when the first protozoa developed. Due to the ubiquitous presence of CK2 in protozoa, yeast, plants, and animals, this enzyme seems to play an important role in living organisms.

This important role of protein kinase CK2 is supported by the fact that up to now more than 160 substrates have been identified (Pinna and Meggio 1997). These substrates are involved in the regulation of transcription, signal transduction processes, growth control, various steps of development, and the formation of cellular shape and architecture. In addition to the cellular substrates, a number of viral proteins are phosphorylated by CK2 (Guerra and Issinger 1999). There is now accumulating evidence for the presence of individual subunits of CK2, in addition to the subunits in the holoenzyme. Moreover, there are now an increasing number of proteins binding exclusively to the catalytic α- or the regulatory β-subunit (Glover 1998; Guerra and Issinger 1999).These interactions can lead to an alteration of the enzymatic activity of the kinase itself (Götz et al. 1996; Guerra et al. 1997; N. Schuster, C. Götz, E. Schneider, A. Prowald, M. Faust, M. Montenarh, unpublished work), as well as of its binding partners such as the tumor suppressor p53, protein phosphatase 2A, and topoisomerase (Hériché et al. 1997; Prowald et al. 1997; Redwood et al. 1998; Schuster et al. 1999). Although the number of new, interacting partners of protein kinase CK2 is increasing rapidly, we are still in the early stages of understanding the regulation and the cellular function of this kinase.

Further progress in CK2 research has been made by the crystallization of the catalytic (Guerra et al. 1998; Niefind et al. 1998) and the regulatory (Chantalat et al. 1999) subunits and by the analysis of the three-dimensional structure. There are a lot of recent reviews dealing with substrates of CK2, its possible regulation, and implication in cell proliferation, as well as with its structure-function relationship (Allende and Allende 1995, 1998; Guerra and Issinger 1999; Guerra et al. 1999a; Pinna 1997; Pinna and Meggio 1997). However, an important aspect that has not been considered adequately is the tissue distribution and cellular and subcellular localization of protein kinase CK2, which might also contribute to its various functions and to its regulation. In this review, we will show in which organisms protein kinase CK2 is found, in which tissues the kinase is highly expressed, and in which subcellular compartments this kinase is localized, and we will address the functional relevance of this localization.

Pedigree of protein kinase CK2

So far protein kinase CK2 has not been detected in *Eubacteria* or *Archaebacteria*. But CK2 has been found in very early eukaryotes such as *Theileria parva* and in *Dictyostelium discoideum* (Kikkawa et al. 1992; Ole-Moyoi et al. 1992; Ospina et al. 1992). The protozoans *Theileria parva* and *Dictyostelium discoideum* (Hanks and Quinn 1991) only express the catalytic α-subunit, lacking the regulatory β-subunit. The amino acid sequence of the CK2 catalytic α-subunit of *Theileria parva* shows 68% identity with the *Drosophila melanogaster* α-subunit and 67% with the *Caenorhabditis elegans* α-subunit, but only 58% and 56% sequence identity with the *Saccharomyces cerevisiae* α- and α´-subunits. The amino acid sequence identity of the catalytic subunit of *Dictyostelium discoideum* (Hanks and Quinn 1991) with *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* α-subunits is 62% and 51%, respectively, but there is a higher identity (57%) with the catalytic α´-subunit of *Saccharomyces cerevisiae* (Guerra and Issinger 1999; Guerra et al. 1999a, 1999b). As plants, animals, and mushrooms evolved from primitive metazoa, protein kinase CK2 remained conserved and has been transmitted. Furthermore, CK2 is also found in *Arabidopsis thaliana* and *Zea mays*, representatives of the plant kingdom (Collinge and Walker 1994; Dobrowolska et al. 1991; Mizoguchi et al. 1993). *Arabidopsis thaliana*, the mouse-ear cress, has two catalytic α-subunits and four regulatory β-subunits, whereas *Zea mays* possesses only one catalytic α-subunit of CK2 (Dobrowolska et al. 1991).

CK2 is found in three groups of yeasts. *Schizosaccharomyces pombe* (Roussou and Draetta 1994) expresses only one catalytic α- and one regulatory β-subunit, whereas *Saccharomyces cerevisiae* has two different catalytic and regulatory subunits (Bidwai et al. 1992, 1994, 1995). The third member of the group is the deuteromycete *Candida albicans* (Walz et al. 1997), exhibiting the same CK2 subunit distribution as *Schizosaccharomyces pombe*. In the animal kingdom, protein kinase CK2 is found in nematodes (*Caenorhabditis elegans*, accession No.: P28548, O62352, Q22077, P18334), insects (*Drosophila melanogaster*, *Spodoptera frugiperda*, Acc. No.: P08182, O96863, P08181, O76485), amphibians (*Xenopus laevis*, Acc. No.: P28021, P28020), fish (*Brachydanio rerio*, Acc. No. Q91398), birds (*Gallus gallus*), and mammals (*Homo sapiens*, *Bos taurus*, *Sus scrofa*, *Oryctolagus cuniculus*, *Rattus norvegicus*, *Mus musculus*, etc., Acc. No.: P13862, P07312, P19138, P19139, P33674, Q60737, P21868, P21869, P19784, P20427). Presumably, ever since the first eukaryotes (the protozoa) developed and were then distributed in all branches of the eukaryotic pedigree, protein kinase CK2 has been an important factor in the survival of eukaryotic life.

Tissue distribution of protein kinase CK2

CK2 is not only distributed in various species at different stages of development, almost all tissues of higher organisms express CK2. The distribution of CK2 in different organs has been analyzed by different methods: (1) at the mRNA level by northern blot and/or in situ hybrid-

ization; (2) at the protein level by Western blot analysis and/or immunohistochemistry; or (3) by kinase activity assays. In 1994 Mestres et al. investigated the mRNA level CK2 α- and CK2 β-subunits by in situ hybridization and CK2 transcripts, and both CK2 protein subunits were detected in nearly all organs of the mouse embryo, suggesting a more general role during embryonic development. In general the level of CK2 transcripts correlates with the protein expression profile. In the skin, however, these authors have detected a high level of transcripts but low protein expression. In the same study, by immunohistochemistry using CK2 α- and CK2 β-specific antibodies, it was shown that during mouse embryogenesis CK2 was predominantly expressed in neuroepithelia at postcoital day 10.5 (PC10.5). CK2-specific labeling of neural structures by in situ hybridization and immunohistochemistry was also seen during late stages of development. Together with earlier data (Diaz-Nido et al. 1992), these findings led to the suggestion of a brain-specific role for CK2 in the regulation of neuronal functions. From PC11.5 in all epithelia, high expression of CK2 is observed. From PC16.5 onward, all tissues and *anlagen* that are involved in organogenesis show high levels of CK2 expression. Diaz-Nido et al. (1994) have reported that protein kinase CK2 is very abundant in rat brain when compared with other rat tissues. By using antibodies against peptides that are unique for the α- or the α′-subunit, a significant increase in the amount of the α′-subunit during the late postnatal neocortical maturation period is observed. Furthermore, the α′-subunit is much more abundant in neurons than in glia cells and therefore one might speculate that the isoform of the holoenzyme of CK2 containing the α′-subunit alone may have some functions in fully differentiated neurons.

By analyzing the nuclear CK2 activity in different tissues, the highest activity has been found in testis, brain, and liver, whereas CK2 activity in kidney and spleen is low (Yutani et al. 1982). These data are only partly confirmed (Singh and Huang 1985), since these authors have found that the CK2 activity is very strong in rat spleen and testis, and low in liver, kidney, lung, and brain. Very low activity is found in skeletal muscle and adipose tissue. By analyzing the CK2 activity in the cytosolic fractions of different tissues, low CK2 activity is found in skeletal muscle, cardiac muscle, and adrenal gland, and a higher activity in lung, spleen, testis, and thymus (Nakajo et al. 1986). Girault et al. (1990) have analyzed the CK2 activity in brain, especially in cerebral cortex, hippocampus, and caudate putamen, where they found a high CK2 activity compared with a low activity in liver, spinal cord, and spleen. Recently, the observation that the highest CK2 activity is found in testis and brain has been confirmed for mouse. In addition this report has demonstrated that the protein kinase CK2 activity is directly correlated with the expression level of the CK2 α- and CK2 β-subunit (Guerra et al. 1999b). In Western blot analysis, Guerra et al. (1999b) have shown that CK2α detection is strongest in testis and brain, followed by spleen, liver, and lung; the lowest amounts are found in heart and kidney. The CK2 α′-subunit is only expressed in testis and brain. The CK2 β-subunit is strongly detected in testis and brain, followed by spleen. These results have been confirmed and extended by Western blot analysis, which shows that CK2α′ and CK2β are preferentially expressed in adult testis, sperm, and brain (Xu et al. 1999). In situ hybridization studies have demonstrated that CK2α is highly expressed in spermatogonia and then decreases when cells differentiate, declining in postmeiotic, round spermatids, with little or no signal seen in the later elongating or elongated spermatids. CK2α′ mRNA is low in spermatogonia, but preferentially expressed in more differentiated sperm cells. CK2β is abundantly and broadly expressed, but increases in differentiating germ cells.

Subcellular localization of protein kinase CK2

In the early reports, no differences in CK2 activity were detected when CK2 was used from cytosol or nucleus. However, it turned out that CK2 was present not only in the nucleus and the cytoplasm but nearly everywhere in the cell. The production of new antibodies specific for individual subunits of CK2 made the analysis of the subcellular localization of CK2 easier and more meaningful.

Plasma membrane

Detection of protein kinase CK2 activity in plasma membrane preparations has been reported by various authors (Pyerin et al. 1986; Sargiacomo et al. 1994; Skubitz et al. 1991; Wei and Tao 1993). Oligomeric forms of CK2 are closely associated with highly purified rat liver plasma membrane preparations and plasma membranes prepared from A431 cells and from SF9 insect cells expressing the catalytic α- and the regulatory β-subunits of CK2 (Sarrouilhe et al. 1998). The holoenzyme seems to be targeted to the plasma membrane by the β-subunit of CK2. The same region of the β-subunit of CK2 that is responsible for binding to polyamines also mediates binding to the plasma membrane. Analysis of the association of CK2 with the plasma membrane during rat development has revealed that the level of CK2 is low in prenatal stages, but an increase is observed within the first week after birth. Based on these observations, one has to conclude that the plasma membrane localization of CK2 is developmentally regulated. The topographical orientation of the protein kinase within the plasma membrane has not always been determined and therefore it is impossible to assess the membrane-bound form of CK2 as an ectoprotein kinase or a peripheral membrane protein exposing its catalytic domain toward the cytoplasm. Wei and Tao (1993) have shown CK2 activity in membrane and cytosolic preparations of human erythrocytes. Their analysis shows that both membrane-bound and cytosolic CK2 phosphorylate most of the cytoskeletal proteins, including spectrin, ankyrin, and adducin; and CK2 is un-

able to phosphorylate actin. These data suggest that CK2 may play a role in the regulation of cytoskeletal protein interaction. CK2 is not only present in the plasma membrane in its free form but also associated with other proteins.

Raman et al. (1998; Raman and Kimberly 1998) have reported that the regulatory β-subunit of CK2 interacts specifically with the cytosolic domain of the transmembrane protein CD5, and CK2 phosphorylates CD5 at serine 459 and serine 461. CD5 is expressed on all T cells and the B1 subset of B cells, and it modulates antigen receptor-mediated activation. Furthermore, cross-linking experiments with anti-CD5 antibodies leads to the activation of CD5-associated CK2 in a murine B-lymphoma cell line and human T-leukemia cell line. Raman's reports (Raman and Kimberly 1998; Raman et al. 1998) demonstrate for the first time the localization of CK2 at the cell membrane in association with a cell surface receptor, and suggest that CK2 may play a role in the regulation of membrane proximal signaling events. An extracellular ectoprotein kinase that phosphorylates the complement component C9 in human leukemia cells is related to protein kinase CK2 (Paas et al. 1999). Furthermore, a membrane preparation containing endoplasmin has exhibited protein kinase activity. Substrate specificity and inhibition by heparin are characteristic for CK2. Moreover, the associated protein kinase phosphorylates endoplasmin at the same positions as exogenously added CK2 (Trujillo et al. 1997). In 1986 Pyerin et al. demonstrated that a highly purified ectoprotein kinase from HeLa cells fulfilled all characteristics of protein kinase CK2. Meanwhile, the possibility that CK2 acts as an ectoprotein kinase that is associated with the outer surface of the plasma membrane has been described by several groups. Walter et al. (1996) have identified *ecto*PKII as a protein kinase CK2-like enzyme. This kinase phosphorylates the CK2-specific peptides RRREEETEEE and RRRAADSDDDDD, is inhibited by low concentrations of heparin, and uses both ATP and GTP as cosubstrates. This classification has been confirmed by further characterization, including enzyme autophosphorylation data that shows two (43 kDa and 40 kDa) catalytic subunits and a 28-kDa noncatalytic subunit, immunological reactions to specific human anti CK2 antibodies, tryptic peptide maps that result in comparable fragmentation of *ecto*PKIIα, and authentic human CK2 α-subunit, mass spectrometry of separated tryptic peptides from *ecto*PKIIα and CK2α, and, finally, microsequencing. This ectoprotein kinase phosphorylates some physiological extracellular substrates such as fibrinogen, fibrin (Sonka et al. 1989), vitronectin (Skubitz et al. 1991), lectin L-29 (Huflejt et al. 1993), and neurochordins (Elizarov and Preobrazhensky 1993). CK2 is found not only on the cell surface but also on shed microparticles (Nilsson Ekdahl and Nilsson 1997). Dot-blot analysis is used to determine the existence of CK2 on microparticles. FACS analysis of intact U937 cells with monoclonal antibodies against CK2 has shown its localization on the cell surface. Using laser scanning and immunoelectron microscopy, a recent study has demonstrated that protein kinase CK2 and nucleolin are located on the cell surface, where they colocalize with the urokinase receptor (uPAR; Dumler et al. 1999). Moreover, these proteins are cointernalized into the cell as an intact complex. Immunoprecipitation experiments combined with kinase assays show a specific association of uPAR with nucleolin and CK2 and have revealed a urokinase (uPA)-induced activation of CK2, which causes the phosphorylation of nucleolin. Blockage of nucleolin and CK2 through specific modulators leads to the inhibition of uPA-induced cell proliferation. Cell surface nucleolin has been reported to bind growth factors, laminin, lipoproteins, the complement inhibitory factor J, and several viruses (De Verdugo et al. 1995; Kibbey et al. 1995; Larrucea et al. 1998; Semenkovich et al. 1990). These findings raise the possibility that the interaction between nucleolin and CK2 might represent a signaling mechanism that modifies the cellular mitogenic response.

All in all, protein kinase CK2 is a plasma membrane-associated enzyme with extracellular as well as intracellular binding partners or substrates. Phosphorylation of these substrates or the binding to plasma membrane-associated proteins seems to be implicated mainly in the regulation of cell proliferation or immune surveillance.

Cytoplasm

Using subcellular fractionation experiments, many groups have detected protein kinase CK2 in the cytoplasm (Girault et al. 1990; Goueli et al. 1986; Kandror et al. 1989; Singh and Huang 1985). Yu et al. (1991) have studied the localization of CK2 by immunofluorescence and immunoelectron microscopy in HeLa cells. Their results indicate that the CK2 α- and CK2 β-subunits are localized in the cytoplasm during interphase and are distributed throughout the cell during mitosis. In contrast, the CK2 α′-subunit is localized in the nucleus during the G_1-phase and in the cytoplasm during the S-phase. These results suggest that CK2 may play a significant role during cell division by shifting its localization between the cytoplasm and the nucleus. Moreover, these results also indicate an asymmetrical distribution of the different subunits of CK2. Diaz-Nido et al. (1992) have shown that the level of cytosolic protein kinase CK2 is elevated in serum-starved neural NIA-103 cells that are treated with DNA synthesis inhibitors. It was supposed that the high cytosolic levels of CK2 led to a better phosphorylation of the microtubule-associated protein 1B (MAP-1B) followed by neurite outgrowth. A shift to an elevated cytosolic concentration of CK2 in nonproliferating, differentiating neuroblastoma cells is consistent with its putative role in the regulation of the cytoskeletal rearrangements underlying neuronal morphogenesis and plasticity. The abundance of CK2 in the cytoplasm of neurons, but not in glia cells, has been confirmed by immunohistochemistry (Girault et al. 1990; Iimoto et al. 1990). By using antibodies specific for the CK2 α- or β-subunit in mouse

NIH-3T3 cells, the CK2 α-subunit shows a cytosolic localization, whereas the β-subunit is mostly nuclear, confirming the asymmetrical distribution of different subunits within the same cell (Schmidt-Spaniol et al. 1993).

In synchronized normal and tumor cells, the α- and β-subunits are predominantly nuclear and there is no difference between primary and tumor cells. In contrast, in serum-starved human leukemia U937 cells, CK2 is found in the cytoplasm (Watabe et al. 1997). Treatment of these cells with 10^{-8} M bufalin (prepared from toad venom, which selectively inhibits the growth of various tumor cell lines) in the absence of serum results in a translocation of CK2 from the cytoplasm to the nucleus. It is thought that this translocation induces apoptosis through phosphorylation and activation of topoisomerase II by CK2. Another report has shown that CK2 is located in the cytosol as well as in the nucleus (McKendrick et al. 1999). However, all these results indicate that the localization in both compartments, nucleus and cytoplasm, may be dependent on defined cellular conditions and that this localization may vary from one cell type to another. Moreover, localization of CK2 in the cytoplasm or in the nucleus seems to reflect a dynamic process.

Mitochondria

A closer look at the subcytosolic localization of CK2 reveals that this enzyme is also associated with cytosolic organelles. Protein kinase CK2 has been purified from bovine kidney mitochondria (Damuni and Reed 1988). Furthermore, a protein kinase activity has been characterized in liver mitochondria by its ability to phosphorylate a CK2-specific peptide substrate. The CK2 activity is located predominantly in the intermembrane space of quiescent mitochondria. A translocation of the enzyme to the inner membrane of energized mitochondria occurs in the presence of spermine. An in vivo localization of CK2 at the inner membrane seems to be reasonable, since some potential substrates of CK2 are localized in the matrix of mitochondria (Sarrouilhe and Baudry 1996).

Endoplasmic reticulum

Protein kinase CK2 has been identified further as an endoplasmic reticulum (ER)-associated kinase responsible for the in vitro phosphorylation of calnexin and signal sequence receptor-α (SRSα; Ou et al. 1992). SRSα is a major calcium-binding protein of the ER, which is implicated in chaperone functions. Calnexin is a lectin chaperone of the ER that couples temporally and spatially N-linked oligosaccharide modifications with the productive folding of newly synthesized glycoproteins. Calnexin was originally identified as a major type-I integral membrane protein substrate of kinases associated with the ER. The cytosolic domain of calnexin was phosphorylated in vivo, at protein kinase CK2 sites serine 534 and serine 544 and at a protein kinase C/proline-directed kinase site, serine 563 (Wong et al. 1998). It has also been shown that the phosphorylation of the cytosolic domain of calnexin, by concerted actions of protein kinase CK2 (at serine 534 and serine 544) and extracellular signal-regulated kinase-1 (ERK-1) at serine 563, targets calnexin to the ribosomes (Chevet et al. 1999). Conditions that were expected to enhance calnexin binding near the translocon (i.e., overexpression of calnexin by transient transfection) have been shown to increase the productive folding of the cotransfected subunit of the nicotinic acetylcholine receptor (Chan et al. 1997). The CK2-mediated and ERK-1-mediated enhancement of calnexin association with ribosomes provides a regulatory mechanism to increase the glycoprotein-folding capacity near the translocon.

Cytoskeleton

Serrano et al. (1989) has shown that CK2 is associated with microtubules and appears enriched in cold, stable microtubule fractions from rat brain. Furthermore, in vitro binding experiments have shown that CK2 binds preferentially to MAP-1A, MAP-1B, and MAP2. Immunofluorescence analysis of normal rat kidney (NRK) cells and kidney fibroblasts has revealed that anti-CK2 antibodies stain some microtubule fibers and, in dividing cells, the mitotic spindle. In cultures of proliferating neuroblastoma cells, nuclear staining for CK2 is more intense than in NRK cells. In differentiated neuroblastoma cells, predominant cytoplasmic staining and colocalization of CK2 and tubulin in neurites has been observed. These data indicate a relocalization of CK2 upon switching from proliferation to differentiation. The association with the mitotic spindle has been confirmed and extended in as much as all three subunits of CK2, α, α′, and β, are bound to the spindle apparatus (Krek et al. 1992). Further, it is known that CK2 phosphorylates β-tubulin in vitro and in vivo (Crute and van Buskirk 1992; Kohtz and Puszkin 1989; Serrano et al. 1987). Other cytoskeletal substrates of CK2 are myosin heavy and light chains (Pinna and Meggio 1997), troponin-T, τ-protein (Greenwood et al. 1994), MAP-1B (Allende and Allende 1995), and dynein (Karki et al. 1997). CK2 also modifies stathmin at its residue 146 and slightly increases the ability of stathmin to depolymerize microtubules (Moreno and Avila 1998). Phosphorylation of tubulin by CK2 enhances its binding to the neuronal protein NP185, which is associated with brain-coated vesicles (Kohtz and Puszkin 1989). However, tubulin is not only a substrate for CK2 but also binds to the catalytic α- and α′-subunit (Faust et al. 1999). Faust's is also one of the first reports describing a protein that is bound to the α′-subunit. One can suppose that a stable binding between CK2 and microtubles can localize 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 it has been suggested that CK2 plays a role in cell polarity

and differentiation. In nonproliferating, differentiating neuroblastoma cells, CK2 shifts to an elevated cytosolic concentration, which is consistent with its regulation of the cytoskeletal rearrangements underlying neuronal morphogenesis and plasticity (Diaz-Nido et al. 1992). Furthermore, the gene for the catalytic α-subunit of CK2 in *Schizosaccharomyces pombe* has a temperature-sensitive, recessive lethal mutation (*orb5*), which confers a spherical morphology to the filamentous yeast (Snell and Nurse 1999). Deletion of the gene for the regulatory β-subunit of CK2 in *Schizosaccharomyces pombe* is not lethal but shows also an abnormal, rounded morphology reminiscent of *orb5* (Roussou and Draetta 1994). The temperature-sensitive mutation (cka1^{ts}) of CK2 in *Saccharomyces cerevisiae* showed, after shift to the restrictive temperature, a population of budded and unbudded cells with spherical morphology (Rethinaswamy et al. 1998). In neuroblastoma cells, where the activity of CK2 was deleted through antisense technology, neuritogenesis and cell polarity were inhibited (Ulloa et al. 1993).

CK2 activity, as well as the concentration, is reduced in Alzheimer's, Pick's, and Biswanger's disease (Iimoto et al. 1990). The distribution is altered in that CK2 is no longer found in axons. Instead, CK2 is found in both the fibrillar soma and dendrites. Furthermore, neurofibrillary tangles (NFT) are intensely stained with an antiserum against CK2 in brain tissue of Alzheimer's disease patients (Baum et al. 1992). NFT are pathological cytoskeletal structures composed of paired helical filaments and are found in neurons of patients afflicted with many neurodegenerative disorders.

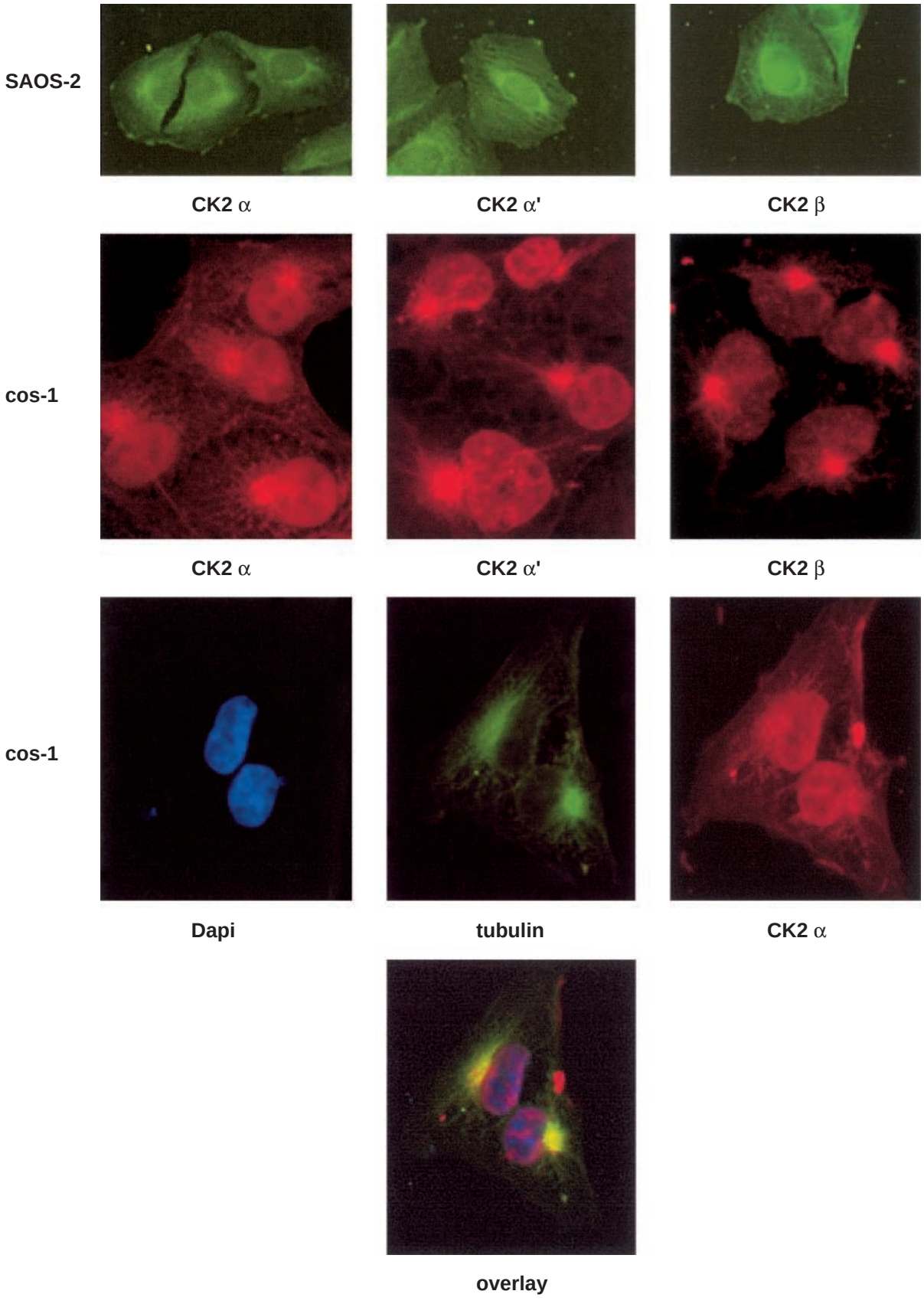
Centrosomes

Centrosomes were discovered as cellular organelles more than 100 years ago by Theodor Boveri. The mammalian centrosome consists of two barrel-shaped centrioles surrounded by a fibrous meshwork termed the pericentriolar material. The best-known function of the centrosome is its ability to nucleate the growth of microtubules. Consequently, the centrosome is essential during interphase for intracellular organelle transport, cell migration, and the establishment of cell shape and polarity. In mitosis it undergoes a dramatic reorganization to form the mitotic spindle. Spindle assembly and organization is orchestrated in part by the centrosome. Protein phosphorylation is one of the key mechanisms that controls the functions of the centrosomes during the cell cycle. There are many kinases known that are associated with the centrosomes, e.g., Cdk1, which is involved in centrosome maturation, polo-like kinases, NIMA-related kinases, and aurora-related kinases. If protein kinase CK2 is also implicated in centrosome function, the processes have yet to be elucidated (Mayor et al. 1999). Recently, depending on the antibody used, intense, weak, or no staining of the centrosomes for CK2α was observed in mouse and rat cells, indicating the presence of immunologically distinct subclasses of CK2α at the centrosomes (McKendrick et al. 1999). In contrast to this finding, a previous, well-controlled study in chicken cells showed that CK2β, but not CK2α, is located at the centrosomes (Krek et al. 1992). By using newly developed immunopurified anti-peptide antibodies against unique regions of CK2α or -α′ and -β (Faust et al. 1999), we found α-, α′-, and β-subunits associated with centrosomes in monkey and human cells (Fig. 2). However, since only the α′- and α-subunits and not the β-subunit bind to tubulin, one might argue that the interaction of CK2 with the centrosomes may be via the catalytic α- and α′-subunits. It would be useful to elucidate the role of CK2 in mitotic control.

Nucleus

As already mentioned for the cytosolic localization of CK2, the reports on nuclear localization are contradictory. Protein kinase CK2 phosphorylates many nuclear substrates, enzymes that are involved in nucleic acid synthesis, transcription factors, nuclear oncoproteins, tumor suppressor proteins, and nucleolar proteins. There are many groups who have found CK2 exclusively located in the nucleus (for review, see Issinger 1993). Others have shown that CK2 is predominantly localized in the nucleus of SCCHW-tumor cells and infiltrating lymphocytes, whereas a more predominant cytosolic staining pattern is observed for normal oropharyngeal mucosa of these tumor patients. In cultures of proliferating mouse neuroblastoma cells, nuclear staining with an anti-CK2 antibody is very intensive. Interestingly, in differentiated neuroblastoma cells, a predominant cytosolic staining is observed (Diaz-Nido et al. 1992; Serrano et al. 1989). These earlier studies, indicating that the nuclear localization of CK2 seems to be a dynamic process, have been confirmed and extended, where it has been shown that CK2α becomes enriched in the nucleus at the late G_1-/early S-phase of the cell cycle and remains there for the rest of the cell cycle (McKendrick et al. 1999). Other authors have reported that CK2 enters the nucleus when cells reenter the cell cycle (Filhol-Cochet et al. 1994). In another report, at quiescence the CK2 β-subunit was reduced in primary cells, whereas the CK2 α-subunit did not change significantly throughout the cell cycle. Furthermore, there seems to be a cell type-specific localiza-

Fig. 2 Indirect immunofluorescence analysis of the individual subunits of CK2 at centrosomes. Subconfluent growing SAOS-2 and COS-1 cells fixed with 2% formaldehyde and permeabilized with 0.2% Triton-X100 were analyzed with rabbit sera against the individual CK2 α-, α′-, and β-subunits in a dilution of 1:100. These antibodies were immune-affinity purified and tested in blocking experiments. Centrosome staining was carried out with mouse monoclonal antibodies against α-tubulin. Tetramethylrhodamine isothiocyanate (TRITC)- and fluorescein isothiocyanate (FITC)-conjugated anti-mouse/rabbit antibodies were used as secondary antibodies. Nuclear staining was performed with 4′,6-diamidine-2′-phenylindole dihydrochloride (Boehringer Mannheim) according to the manufacturer's instructions ▶


SAOS-2
CK2 α
CK2 α'
CK2 β
cos-1
CK2 α
CK2 α'
CK2 β
cos-1
Dapi
tubulin
CK2 α
overlay

tion of the CK2 α-subunit in the cytoplasm or in the nucleus (Schmidt-Spaniol et al. 1993). Yu et al. (1991) have found both subunits in the cytoplasm of HeLa cells, although they used the same approach as the other authors to detect the individual subunits. By using primary human fibroblasts, CK2β was localized predominantly in the nucleus (Lorenz et al. 1993). Both the α- and α´-subunits of CK2 harbor a putative nuclear localization signal (Issinger 1993), which may facilitate the cytoplasmic nuclear transport, whereas the β-subunit lacks such a nuclear localization signal. However, the β-subunit of CK2 may be targeted to the nucleus by binding to NOPP140 (Li et al. 1997).

Nuclear matrix

The nuclear matrix consists of the nuclear lamina, the nucleolus, and the fibrillogranular network. Salt extraction procedures and DNase digest are required to remove nuclear proteins and nucleotides from the nucleus and to prepare the nuclear matrix. Aberrant nuclear and cellular structures are indicators for malignant transformation. Changes in the shape of the nucleus and the cytoskeleton perturb the nuclear matrix (Barboro et al. 1996). These alterations in nuclear shape contribute to changes in DNA synthesis and gene expression (Benecke et al. 1978; Gospodarowicz et al. 1978). Thus, the nuclear matrix plays a fundamental role in replication, transcription, and RNA processing and transport. After response to androgen or certain growth stimuli, protein kinase CK2 translocates from the cytosol to the nucleus, where it associates preferentially with the chromatin and the nuclear matrix (Guo et al. 1999). Three different standard methods for nuclear matrix preparation were compared and quantitatively similar results were obtained for the stimulus-mediated modulation of nuclear matrix-associated CK2 (Tawfic et al. 1997). Furthermore, androgen deprivation in rats led to a decreased activity and concentration of CK2 in the nuclear matrix (about 80%); but, after androgen administration in these deprived rats, CK2 activity and levels increased again in nuclear matrix fractions (110%; Tawfic and Ahmed 1994). The regulatory β-subunit seems to be anchored by covalent sulfhydryl interactions to nuclear matrix components (Zhang et al. 1998). Knockout of the catalytic α´-subunit of protein kinase CK2 in mice causes globozoospermia. Their sperms possess an aberrant morphology and a misshapen nuclear matrix (Xu et al. 1999). Although only little is known about substrates of CK2 at the nuclear matrix, CK2 seems to be an important modulator of the maintenance of the nuclear architecture via phosphorylation and perhaps protein-protein interaction.

Nucleolus

The nucleoli are also part of the nuclear matrix. Pfaff and Anderer (1988) first demonstrated an accumulation of protein kinase CK2 in the nucleolus. With antibodies against CK2, they showed by immunofluorescence analysis an intensive staining of nucleoli in mouse tumor cells. Nucleolar substrates of CK2 are nucleolin, nucleolar protein B23, and p120 cell proliferation nucleolar protein (for review, see Allende and Allende 1995). Furthermore, these nucleolar phosphoproteins were identified as high-affinity substrates for protein kinase CK2 activity (Pfaff and Anderer 1988). Belenguer et al. (1989) detected CK2 by immunocytochemistry in the cytoplasm, nuclei, and nucleoli of growing cells, while it was no longer present in the nucleoli of confluent cells. Isolated nuclei from confluent cells have only a very reduced protein kinase activity with regard to the phosphorylation of the nucleolar protein nucleolin. Reduction of the kinase activity concurs with a highly reduced transcription of rDNA. Thus, the prescence of CK2 in the nucleolus as well as its kinase activity seems to be a key event in the regulation of rDNA transcription.

Nucleosomes

CK2 was also associated with nucleosomal fractions of normal rat prostate. The levels of CK2 activity were higher in active than in inactive nucleosomes. CK2 associated with inactive nucleosomes declined to a minimal level on androgen deprivation, but increased rapidly on androgen administration. Guo et al. (1998) suggested a role for CK2 in promoting the conformational transition of inactive nucleosomes to the active form and in the function of transcriptionally active nucleosomes.

Conclusions

Protein kinase CK2 is not only ubiquitously expressed in nearly every tissue of eukaryotes, it is also found in nearly every compartment of eukaryotic cells (Table 1). In early reports, CK2 was identified by its protein kinase activity, whereas more recently individual CK2 subunits were analyzed immunologically and biochemically with subunit-specific antibodies. By combining both types of analysis, an elevated kinase activity always was found coupled to an elevated level of CK2 and vice versa. However, since with p53 and $p21^{WAF1/CIP1}$ two cellular proteins were identified that regulate the CK2 activity (Götz et al. 1996; Guerra et al. 1997; N. Schuster, C. Götz, E. Schneider, A. Prowald, M. Faust, M. Montenarh, unpublished work), more attempts have to be made to analyze where in the cell these two proteins might be responsible for altered CK2 activity.

Changes in the protein kinase activity of CK2 seem to correlate with cell proliferation where proliferating cells have a higher activity than resting and differentiated cells. Furthermore, elevated activities of CK2 were found in rapidly proliferating tumor cells, whereas low activities were reported for brain preparations of neurodegenerative disorders. Elevated levels of CK2 and cell

Table 1 Immunological detection of CK2

	Anti CK2 antibodies	Anti CK2α antibodies	Anti CK2α´ antibodies	Anti CK2β antibodies
Plasma membrane	Polyclonal serum against purfied CK2 (Dumler et al. 1999)	Monoclonal antibody (Schmidt-Spaniol et al. 1992)		
Cytoplasm		Peptide serum aa 2–22 (Yu et al. 1991)	Peptide serum aa 287–311 (Yu et al. 1991)	Peptide serum aa 140–162 (Yu et al. 1991)
		Pharmacia LKB Biotechnology Uppsala, Sweden (Watabe et al. 1997)	Peptide serum aa 330–349 (Faust et al. 1999)	Peptide serum aa 206–215 (Faust et al. 1999)
		Peptide serum aa 360–371 (Faust et al. 1999)		
Cytoskeleton	Monospecific serum against catalytic subunits (Serrano et al. 1989)	Peptide serum aa 360–371 (Faust et al. 1999)	Peptide serum aa 330–349 (Faust et al. 1999)	Peptide serum aa 206–215 (Faust et al. 1999)
Centrosome		Peptide serum aa 69–87 (McKendrick et al. 1999)	Peptide serum aa 330–349 (Fig. 2)	Peptide serum aa 206–215 (Fig. 2)
		Peptide serum aa 253–269 (McKendrick et al. 1999)		Polyclonal serum against CK2 β (Krek et al. 1992)
		Peptide serum aa 360–371 (Fig. 2)		
Mitotic spindle	Monospecific serum against catalytic subunits (Serrano et al. 1989)	Peptide serum aa 2–22 (Yu et al. 1991)	Peptide serum aa 324–341 (Krek et al. 1992)	Polyclonal serum against CK2 β (Krek et al. 1992)
		Polyclonal serum against CK2 α (Krek et al. 1992)		
Nucleus		Polyclonal serum against CK2 α (Krek et al. 1992)	Peptide serum aa 324–341 (Krek et al. 1992)	Polyclonal serum against CK2 β (Krek et al. 1992)
		Polyclonal serum against CK2 α (Schmidt-Spaniol et al. 1993)	Peptide serum aa 336–350 (Lorenz et al. 1993)	Polyclonal serum against CK2 β (Schmidt-Spaniol et al. 1993)
		Peptide serum aa 329–343 (Lorenz et al. 1993)		Peptide serum aa 55–70 (Lorenz et al. 1993)
Nucleolus	Polyclonal serum against purfied CK2 (Dahmus et al. 1981)			

proliferation seem to correlate with a translocation of CK2 from the cytoplasm to the nucleus. This also indicates that the subcellular localization of CK2 is a dynamic process that changes with the requirement of CK2 at a particular place in the cell. CK2 binds to the ribosomal protein L5 and an interesting question is how the association of CK2 with the ribosomal L5 protein is implicated in the regulation of cell proliferation. CK2 seems also to be essential for the organization of structural elements such as cytoskeleton and nuclear matrix. Loss of CK2α´ in mice results in a misorganization of the nuclear matrix of sperms. Furthermore, depletion of CK2 activity leads to a defect in neuritogenesis. In yeast, CK2 depletion results in a severe change in morphology. The finding that CK2α and-α´ bind to tubulin might indicate that interactions of CK2 with cellular proteins might be necessary to target CK2 to the right place in the cell. This idea is further supported by the finding that CK2 also binds to another cellular protein, namely nucleolin, a protein that is present in the nucleolus, nucleus, cytoplasm, and even on the plasma membrane. Thus, binding of CK2 to nucleolin may support the idea of a specific transport or an anchoring of CK2 to distinct cellular compartments by cellular proteins. A localization of CK2 in association with the plasma membrane may indicate that it is also implicated in immune surveillance and cellular signaling. It is evident that CK2 fulfills most of these diverse functions by phosphorylation of specific substrates in different compartments of the cells. It remains to be elucidated how protein-protein interactions in these various cellular compartments might also contribute to the regulation of its function. Antibodies with new specificities for the individual subunits of CK2 and advanced protein-labeling techniques together with innovative computer-

aided imaging methods will help to solve these problems in the near future.

Acknowledgements The authors would like to thank Drs. Claudia Götz and Norbert Schuster for helpful suggestions and fruitful discussions.

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