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Expression and Localization of Epitope-Tagged Protein Kinase CK2

C. Gail Penner, Zilong Wang, and David W. Litchfield*

Manitoba Institute of Cell Biology, Manitoba Cancer Treatment and Research Foundation, and the Department of Biochemistry and Molecular Biology, University of Manitoba, Winnipeg, Manitoba, Canada

Abstract Protein kinase CK2, formerly known as casein kinase II, is a ubiquitous protein serine/threonine kinase. The enzyme exists in tetrameric complexes composed of two catalytic (CK2α and/or CK2α′) subunits and two subunits (CK2β) that appear to have a role in modulating the activity of the catalytic subunits. With the exception of their unrelated carboxy-terminal domains, the two isozymic forms of mammalian CK2 display extensive sequence identity. Furthermore, CK2α and CK2α′ exhibit remarkable conservation between species, suggesting that they may have unique functions. In the present study, the cDNAs encoding CK2α and CK2α′ were modified by addition of the hemagglutinin tag of the influenza virus at the amino terminus of the respective proteins. The epitope-tagged proteins were transfected into Cos-7 cells and the localization of the expressed proteins determined by indirect immunofluorescence using monoclonal antibodies specific for the epitope tag. The use of transfection favors the formation of homotetrameric complexes (i.e., $\alpha_2\beta_2$, $\alpha'_2\beta_2$) instead of heterotetrameric complexes (i.e., $\alpha\alpha'\beta_2$) that are present in many cells. Epitope-tagged CK2α and CK2α′ displayed kinase activity and the ability to form complexes with CK2β. The results of these studies also indicate definitively that CK2α and CK2α′ are both localized predominantly within the nucleus. Mutation of conserved lysine residues within the ATP binding domains of CK2α and CK2α′ resulted in loss of kinase activity. However, examination of these mutants indicates that kinase activity is not essential for formation of complexes between subunits of CK2 and is not required for nuclear localization of CK2. J. Cell. Biochem. 64: 525–537.

Key words: protein kinase; protein kinase CK2; casein kinase II; casein kinase 2; nuclear localization; epitope tag; expression

Protein kinase CK2 (CK2) is a highly conserved protein kinase that is ubiquitously expressed in eukaryotic cells. Genetic experiments in *Saccharomyces cerevisiae* [Chen-Wu et al., 1988; Padmanabha et al., 1990], in *Schizosaccharomyces pombe* [Snell and Nurse, 1994; Roussou and Draetta, 1994], and in *Dictyostelium discoideum* [Kikkawa et al., 1992] indicate that the enzyme is essential for viability. There are a number of studies suggesting that the activity of CK2 is highly elevated in rapidly proliferating cells and in a variety of tumor and leukemia cells [reviewed by Allende and Allende, 1995; Issinger, 1993]. Furthermore, the recent report of Seldin and Leder [1995] suggests that the dysregulated expression of CK2 can contribute to lymphocyte transformation in transgenic mice. Collectively, these results implicate CK2 as an important component of cellular signalling pathways that control cell proliferation.

From most sources, CK2 is a tetrameric enzyme composed of two α (and/or α′) subunits and two β subunits [reviewed by Pinna, 1990; Litchfield and Luscher, 1993]. The α subunits contain all of the conserved motifs for members of the protein kinase family [Hanks and Quinn, 1991] and exhibit catalytic activity [Hu and Rubin, 1990; Lin et al., 1991; Bodenbach et al., 1994]. The β subunits do not exhibit catalytic activity but are necessary for formation of tetrameric CK2 complexes [Gietz et al., 1995] and appear to modulate the catalytic activity of the α subunits [Filhol et al., 1991; Heller-Harrision

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*Correspondence to: David Litchfield at his present address: Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 5C1.

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and Czech, 1991; Boldyreff et al., 1993]. In a number of organisms, including mammals and birds, a second isozymic form of the CK2 catalytic subunit (designated α′) has been identified [Litchfield et al., 1990; Lozeman et al., 1990; Maridor et al., 1991]. Overall, CK2α and CK2α′ exhibit extensive sequence identity. Within their first 330 amino acids which contain all of the motifs required for catalytic activity, the two isozymes exhibit approximately 90% amino acid identity. By comparison, the carboxy-terminal domains of CK2α (approximately 60 amino acids) and CK2α′ (approximately 20 amino acids) are completely unrelated. Importantly, the unique aspects of CK2α and CK2α′ are highly conserved between chickens and humans; CK2α and CK2α′ exhibit 98% and 97% identity, respectively. Studies from different laboratories have recently demonstrated that CK2 exists in homotetrameric complexes (i.e., $\alpha_2\beta_2$, $\alpha'_2\beta_2$) and in heterotetrameric complexes ($\alpha\alpha'\beta_2$) [Gietz et al., 1995; Chester et al., 1995].

Relatively little is known regarding the individual functions or characteristics of CK2α and CK2α′. An initial indication of potential functional differences between CK2α and CK2α′ came from the studies of Yu et al. [1991], who demonstrated a dramatic difference in the localization of the two isoforms. In interphase cells, these authors concluded that CK2α was predominantly cytoplasmic and CK2α′ primarily nuclear. These observations suggested that the unique carboxy-terminal domains of CK2α or CK2α′ have signals that specify subcellular localization. By comparison, less obvious differences were observed by Krek et al. [1992], who concluded that CK2 is predominantly nuclear. Since the composition of tetrameric complexes was not defined in the latter studies, it is not clear if the similar distribution of CK2α and CK2α′ resulted because the two isozymes were found predominantly within the same tetrameric complexes (i.e., $\alpha\alpha'\beta_2$). In this regard, we and others have previously noted that CK2α′ is much less prominent than CK2α in a number of model systems [Litchfield et al., 1991, 1992; Krek et al., 1992; Luscher and Litchfield, 1994], suggesting that where CK2α′ is less abundant than CK2α, CK2α′ exists predominantly in tetrameric complexes that also contain CK2α.

We undertook the present studies to resolve the disrepancies arising from the two previous studies. Furthermore, based on the observations of Yu et al. [1991], we were interested in defining sequences within CK2α and CK2α′ that define whether these proteins are localized within the cytoplasm or the nucleus. As an initial step towards achieving these objectives, we examined the subcellular localization of epitope-tagged CK2α or CK2α′ using monoclonal antibodies directed against the epitope to detect the expressed proteins. We observed that expressed CK2α and CK2α′ are both localized primarily to the nucleus. Furthermore, based on our observations, we conclude that the carboxy-terminal domains of CK2α and CK2α′ do not influence the cytoplasmic vs. nuclear localization of the proteins. We also demonstrate that kinase activity is not essential for the formation of multisubunit CK2 complexes and is not required for nuclear localization of CK2.

MATERIALS AND METHODS

Materials

Fluorescein isothiocyanate (FITC)–conjugated goat antimouse antibody and tetramethylrhodamine B isothiocyanate (TRITC)–conjugated goat antirabbit were purchased from Sigma (St. Louis, MO). Horse radish peroxidase–conjugated goat antimouse antibody and horse radish peroxidase–conjugated goat antirabbit antibody were purchased from BioRad (Mississauga, Ontario, Canada). Oligonucleotides were obtained from Dalton Chemical (Toronto, Ontario, Canada) or from Gibco BRL (Burlington, Ontario, Canada). Synthetic peptides were synthesized on an Applied Biosystems (Foster City, CA) model 431A peptide synthesizer as previously described [Bosc et al., 1995]. Nitrocellulose and polyvinylidene difluoride (PVDF) membrane were purchased from BioRad. Molecular weight markers were Rainbow™ colored protein molecular weight markers from Amersham (Arlington Heights, IL). SV40-transformed African green monkey kidney cells (Cos-7) were obtained from the American Type Culture Collection (Rockville, MD) and were cultured in α-modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C in the presence of 5% CO_2.

Plasmid Constructs

Manipulation of the DNA was according to Sambrook et al. [1989]. The human α and α′ sequences of protein kinase CK2 (hT4.1 and hT9.1, respectively, in Lozeman et al. [1990] were modified by ligation of the coding sequence for a triplet of the influenza hemagglutanin (HA) epitope (YPYDVPDY [Tyers et al.,

1992]) to the 5′ end of the protein coding region of the respective protein kinase CK2 subunits (Fig. 1). In each case, the polymerase chain reaction (PCR), as described by Innis et al. [1990], was used to create a Not I site in frame with the codons of the HA epitope sequence and with the respective CK2 coding sequences. These primers also deleted the first methionine of the respective CK2 subunits. The primers for CK2α were ATAAGAATGCGGCCGCTCGGGACCCGTGCCA (sense) and ATCATAATTGTCATGTCCATGGAA (antisense) and for CK2α′ were ATAAGAATGCGGCCGCCCCGGCCCGGCCGCG (sense) and CAGGATCTGGTAGAGTTGC (antisense). The sequences generated by PCR extended from the mutated 5′ end of the respective subunit to nucleotide 878 (α) and nucleotide 558 (α′). Amplified fragments were digested with Not I and Nco I (for α) or with Not I and Ppum I (for α′) and subcloned into the Not I/Nco I and Not I/Ppum I sites in the hT4.1 and hT9.1 plasmids. All amplified sequences were verified by DNA sequencing according to the method of Sanger et al. [1977]. Simultaneously, the HA epitope sequence was modified and subcloned into the TA^R Cloning vector, pCR™II from Invitrogen (La Jolla, CA). The HA sequence (GGCCGCATCTTTTACCCATACGATGTTCCTGACTATGCGGGCTATCCCTATGACGTCCCGGACTATGCAGGATCCTATCCATATGACGTTCCAGATTACGCTGCTAGTGCGGCCGC) [Tyers et al., 1992; Egan et al., 1993] was a gift from Dr. Sean Egan (Hospital for Sick Children, Toronto, Canada). To introduce a start codon and a Hind III site at the 5′ end of the tag, we amplified this sequence using the following primers: CCCAAGCTTCCACCATGGGCCGCATCTTTTACCCA (sense primer) and ATAGTTTAGCGGCCGCACTGAGCAGC (antisense primer). After subcloning into the TA vector, the amplified sequences were verified by DNA sequencing. The HA containing TA vector was digested with Not I/Apa I, and the Not I/Apa I fragment of each of the respective CK2 subunits was subcloned into the TA vector. The HA-tagged CK2α or CK2α′ cDNAs, designated HA-α or HA-α′, respectively, were subsequently subcloned into the Hind III site of pRc/CMV (Invitrogen). HA-α/α′ (N-terminal domain of α with the C-terminal domain of α′) and HA-α′/α (N-terminal domain of α′ with the C-terminal domain of α) were generated by swapping the carboxy-terminal domains of the respective proteins. Bsu 36 1 sites at nucleotide 1041 of hT 4.1 and at nucleotide 1,058 of hT 9.1 were used to effect this swap. A myc-epitope tag (MASMEQKLISEEDLNN [Evan et al., 1985]) was ligated to the 5′ end of the coding region of CK2β in the pKS II+ vector (a gift from Dr. Stephane Richard, Washington University, St. Louis, MO). The full coding region of myc-β was obtained by Not 1/Apa 1 digest and was subcloned into the respective sites of pRc/CMV.

Kinase-inactive mutants of HA-α (HA-αk.d.) and HA-α′(HA-α′k.d.) were created by mutating the codon for lysine to methionine at amino acid positions 68 and 69, respectively [Lozeman et al., 1990; Birnbaum and Glover, 1991]. The primers used were TGGCTTGAGAATCATAACAACAACTTTTT and CTGGCTTCAGGATCATTACAACCACTCT for α and α′, respectively. Mutations were generated using the Muta-Gene® M13 In Vitro Mutagenesis Kit, Version 2 (BioRad) and were confirmed by DNA sequencing.

Antibodies

Polyclonal anti-CK2α antiserum was prepared as described in Litchfield et al. [1991] and was directed against the synthetic peptide $\alpha^{376-391}$. Polyclonal anti-CK2α′ antibody directed against the synthetic peptide $\alpha'^{333-350}$ was described previously [Litchfield et al., 1992]. Importantly, these antibodies are directed against peptides within the unique carboxy-terminal domains of the two proteins. The anti-α′ antibody was affinity-purified according to Harlow and Lane [1988] using $\alpha'^{333-350}$ peptide attached to cyanogen bromide–activated Sepharose 4B. The monoclonal antibody 12CA5 which reacts against the HA epitope [Wilson et al., 1984; Field et al., 1988] was purchased from BAbCO (Berkeley, CA). The hybridoma producing the 9E10 monoclonal antibodies directed against the myc epitope [Evan et al., 1985] was injected into mice and ascites fluid was collected and purified by ammonium sulphate precipitation.

Methods

Transfection of cells. Calcium phosphate coprecipitation of DNA was used for transfection of cells [Ausubel et al., 1990]. Cos-7 cells were plated at 5×10^5 cells per 10 cm dish. For smaller plates, all reactions were scaled back to reflect the surface area of the plate. After 24 h, the cells were refed with fresh media. Two to four hours later the cells were transfected with plasmid DNA that had been purified by double

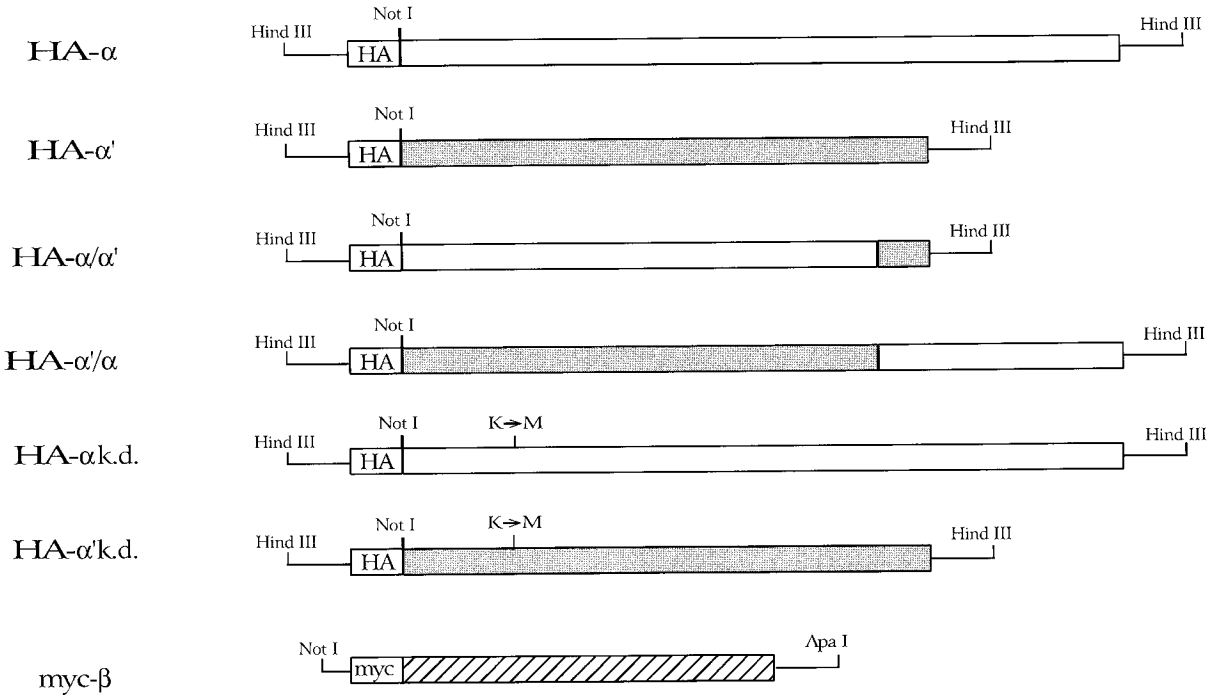

Fig. 1. Epitope-tagged CK2 constructs. Constructs, designated HA-α and HA-α′, encoding CK2α and CK2α′ with the HA epitope at the N-terminus of each of the proteins were prepared as described in Materials and Methods. As described in the text, a Not I site facilitated ligation of the HA-epitope sequence to the cDNAs encoding CK2α and CK2α′. Chimeric proteins, designated HA-α/α′ (N-terminal domain of α with the C-terminal domain of α′) and HA-α′/α (N-terminal domain of α′ with the C-terminal domain of α) were generated by swapping the carboxy-terminal domains of the respective proteins. Kinase-inactive variants of the two proteins, designated HA-αk.d. and HA-α′k.d., were generated by mutation of an essential lysine residue (K68 in CK2α, K69 in CK2α′) within the ATP-binding domain of the respective proteins to methionine. The myc-β construct encodes the myc epitope at the amino terminus of CK2β. Protein coding sequences derived from CK2α are marked by the open boxes, those derived from CK2α′ are marked by the shaded boxes, and those derived from CK2β are marked by the hatched box. The CK2α and CK2α′ constructs were subcloned into the Hind III site of pRc/CMV. Myc-β was subcloned into pRc/CMV using the Not I and Apa I sites. The HA epitope is recognized by 12CA5 monoclonal antibodies and the myc epitope by 9E10 monoclonal antibodies. Polyclonal anti-CK2α antibodies and polyclonal anti-CK2α′ antibodies recognize the carboxy-terminal domains of the respective proteins.

$CsCl_2$ gradient centrifugation. The DNA transfection mix was prepared with 50 µg DNA in 0.5 ml 0.25 M $CaCl_2$ which was added with mixing to 0.5 ml of 2× HBS (0.28 M NaCl, 50 mM HEPES, pH 7.05–7.12, 1.5 mM Na_2HPO_4). The mixture was allowed to stand for 20 min prior to transfecting the Cos-7 cells. Transfection was allowed to proceed for 12–14 h, after which the media was removed and the cells washed once with PBS and refed for 36 h and then used for experiments.

Immunofluorescence. Cos-7 cells were plated onto alcohol-sterilized coverslips and transfected as described above. After 36 h, the cells were washed with phosphate buffered saline (PBS), fixed with 2% formaldehyde in PBS for 20 min, extracted with methanol for 20 min, and then washed three times with PBS at 5 min per wash [Chadee et al., 1995]. The fixed cells were reacted with primary antibody in PBS containing 10% fetal calf serum for 1 h at 37°C and were then washed three times with PBS. Anti-CK2α antisera was used at a dilution of 1/400, affinity-purified anti-CK2α′ antibodies at a 1/10 dilution, and 12CA5 antibodies at a 1/200 dilution. Secondary antibodies, either FITC-conjugated goat antimouse antibody or TRITC-conjugated goat antirabbit antibody, were diluted 1/1,000 in PBS containing 10% fetal calf serum. Reaction with secondary antibody was allowed to continue for 2 h at room temperature in the dark, and coverslips were then washed five times with PBS prior to mounting with 20 µl of mounting reagent (50 mg p-phenylenediamine, 5 ml 1 M Tris, pH 7.4, 37.5 ml glycerol). Visualization was with an inverted fluorescent microscope (model Zeiss Axiovert 35 M, Germany). Control experiments

were performed using peptide competition with each of the antigenic peptides (20 μg peptide/μl antibody). Immunofluorescent experiments in which only secondary antibody, rabbit IgG, or preimmune antialpha rabbit sera were also performed. In the latter instances, significant immunofluorescent signals were not detected.

Immunoprecipitation. For immunoprecipitations, each plate of Cos-7 cells was lysed in 1 ml lysis buffer (50 mM Tris.Cl, pH 7.5, 150 mM NaCl, 1% NP-40, 1% aprotinin, 0.1 mM PMSF) for 30 min on ice, and the cells were scraped off the plates and into microfuge tubes. The samples were centrifuged for 10 min, and the cleared lysates were subjected to immunoprecipitation. Antibody used for immunoprecipitation was 20 μl anti-CK2α or anti-CK2α′ and 30 μl of a 50% slurry of protein A Sepharose (Pharmacia) or 12CA5 monoclonal antibody covalently linked to protein A sepharose using rabbit antimouse antibody as a cross-linker as described in Harlow and Lane [1988]. After incubation for 1 h at 4°C, the protein A Sepharose was isolated by centrifugation and washed three times with lysis buffer. If used for electrophoresis, the samples were ready at this stage. For the kinase assay, the protein A Sepharose was washed three times with kinase buffer (50 mM Tris.Cl, pH 7.4, 10 mM $MgCl_2$, 1 mM dithiothreitol).

Kinase assay. Immunocomplexes were prepared as described in the preceding section. Kinase reactions were performed in 50 mM Tris.Cl, pH 7.5, 250 mM NaCl, 10 mM $MgCl_2$, and 100 μM ATP containing 0.7 μCi γ-^{32}P ATP with and without the addition of 1.5 mM DSD peptide (RRRDDDSDDD [Litchfield et al., 1990]) in a total volume of 35 μl. The reaction was initiated by the addition of enzyme to prewarmed buffer, substrate, and ATP. The reaction was allowed to proceed for 5 min at 30°C, and the reaction was stopped by spotting sample on Whatman P81 paper and washed as described in Litchfield et al. [1990]. Counts were determined by scintillation counting. The values for the reactions lacking peptide were subtracted from the values for the reactions containing peptide.

Immunoblotting. Samples were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis using the buffer system of Laemmli [1970]. The protein was transferred to nitrocellulose or PVDF membrane for 1.5 h at 110 volts in blotting buffer (25 mM Tris, 190 mM glycine, 20% methanol) as described in Towbin et al. [1979]. Blocking was with 3% gelatin in TBS (20 mM Tris, pH 7.5, 500 mM sodium chloride) at room temperature for 1 h. The membrane was washed twice in TBST (0.05% Tween-20 in TBS) and reacted with either anti-CK2α (1/1,000), affinity-purified anti-CK2α′ (1/100), 12CA5 monoclonal antibody (1/500), or 9E10 monoclonal antibody (1/500) in 1% gelatin in TBST for 1 h at room temperature. Peptide competitions were performed using 20 μg peptide/μl of antibody. The membrane was washed three times with TBST. Secondary antibody was goat antirabbit or goat antimouse antibody conjugated with horse radish peroxidase diluted 1/25,000 with 1% gelatin in TBST. After reaction for 1 h at room temperature, the membrane was washed five times with TBST. Visualization was with the enhanced chemiluminescence kit (Amersham) according to the manufacturer's recommendations.

RESULTS

Characterization of Anti-CK2α and Anti-CK2α′

Immunoblot analysis was utilized to assess the specificity of the anti-CK2α and anti-CK2α′ antisera (Fig. 2A). With extracts of Cos-7 cells, anti-CK2α antibody (Fig. 2A) recognizes a single protein of approximately 45 kDa that is competed using the peptide $\alpha^{376–391}$, indicating that the antibody is specific for CK2α. Similarly, affinity-purified anti-CK2α′ exhibits specificity for CK2α′ (Fig. 2B). This antibody recognizes a single protein of approximately 40 kDa that is competed using $\alpha^{333–350}$ peptide, consistent with recognition of CK2α′. Importantly, no cross-reactivity between CK2α and CK2α′ is observed with these reagents.

Expression of HA-α and HA-α′

Cos-7 cells were transfected with pRc/CMV encoding HA-α or HA-α′ in the presence of myc-β. Immunoprecipitates using anti-α or anti-α′ antibodies were prepared from lysates of these cells and analyzed on immunoblots using 12CA5 monoclonal antibodies to detect HA-tagged proteins (Fig. 3). It is apparent that HA-α was expressed at much lower levels than HA-α′ (Fig. 3, lanes 1 and 2, respectively). The different levels of expression do not result from differences in immunoprecipitation efficiency, since total lysates of transfected Cos-7 cells also showed a similar lower expression of HA-α than

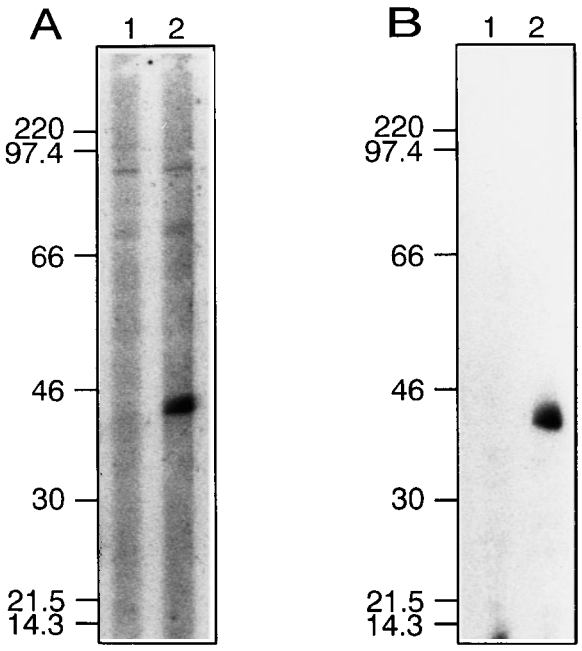

Fig. 2. Specificity of anti-CK2α and anti-CK2α′ antibodies. Total cellular extracts of Cos-7 cells were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to PVDF membrane. **A:** The membranes were probed with anti-CK2α antiserum in the presence (*lane 1*) or absence (*lane 2*) of antigenic $\alpha^{376-391}$ peptide. **B:** The membranes were probed with affinity-purified anti-CK2α′ antibodies in the presence (*lane 1*) or absence (*lane 2*) of antigenic $\alpha'^{333-350}$ peptide.

HA-α′ (data not shown). In order to determine whether the different expression levels of the two proteins resulted from their divergent C-termini, the C-terminal domains of the two proteins were swapped, generating two chimeras, HA-α/α′ and HA-α′/α. Since anti-CK2α and anti-CK2α′ recognize the unique C-terminal domain of the respective proteins, anti-CK2α′ was used to detect HA-α/α′ and anti-CK2α was used to detect HA-α′/α. Protein levels of HA-α/α′ remained low relative to HA-α′/α (Fig. 3, lanes 3, 4), suggesting that the C-terminal domains of the two proteins are not directly responsible for the observed differences in levels of expression. Furthermore, the level of expression of HA-α/α′ was similar to that of HA-α, while the expression of HA-α′/α was similar to that of HA-α′. These results were not due to a random mutation of the parent pRc/CMV plasmid, since replacement of the α′ sequence with the α sequence in HA-α did not result in decreased expression of HA-α′ protein (data not shown). At present, we do not know why HA-α and HA-α′ are expressed to different levels in Cos-7 cells. We also examined the expression of HA-αk.d. and HA-α′k.d. As with other constructs, the level of expression of kinase-inactive HA-α was lower than that of the kinase-inactive HA-α′. The expression level of each of the kinase-inactive variants was similar to that of the respective parent protein.

To further analyze the expressed proteins, we measured kinase activity in 12CA5 immunoprecipitates (Fig. 4). Cos-7 cells were transfected with the plasmids encoding HA-α, HA-α′, or their derivatives with or without the plasmid encoding myc-β. Immunoprecipitates using 12CA5 antibody were prepared and used in kinase assays. Figure 4A shows the results for HA-α′ and its derivatives, and Figure 4B shows the results for HA-α and its derivatives. A low level of ^{32}P-phosphate incorporation was observed in immunoprecipitates prepared from cells that had been transfected with pRc/CMV and myc-β only (Fig. 4A,B). Kinase activity in 12CA5 immunoprecipitates from cells transfected with HA-α′ in the absence of myc-β was approximately thirtyfold above background (Fig. 4A). Coexpression of myc-β with HA-α′ resulted in a further increase in measured kinase activity, consistent with the previous findings of Heller-Harrison and Czech [1991], who examined the expression of CK2α and CK2β in Cos-1 cells. In the presence of myc-β, the chimeric HA-α′/α exhibited similar activity to that of HA-α′. Compared to the other HA-α′ constructs, HA-α′k.d. displayed much less kinase activity, a result consistent with the loss of activity with the K68M mutation [Birnbaum and Glover, 1991]. Since the holoenzyme protein kinase CK2 is a tetramer containing two catalytic subunits, the existence of low levels of kinase activity in the latter anti-HA immunoprecipitates may result from complexes that contain one HA-α′k.d. and one endogenous CK2α or CK2α′.

Analysis of each of the HA-α constructs yields similar observations to those noted with each of the HA-α′ constructs. Kinase activity is displayed by HA-α (Fig. 4B). Furthermore, as is the case with HA-α′, enhanced kinase activity is observed when HA-α is expressed together with myc-β. Kinase activity exhibited by the chimeric construct HA-α/α′ is similar to that of HA-α, while no activity above background is observed with HA-αk.d. Overall, it is apparent that the levels of 12CA5 precipitable kinase activity present in cells transfected with HA-α′ and its derivatives are significantly higher than

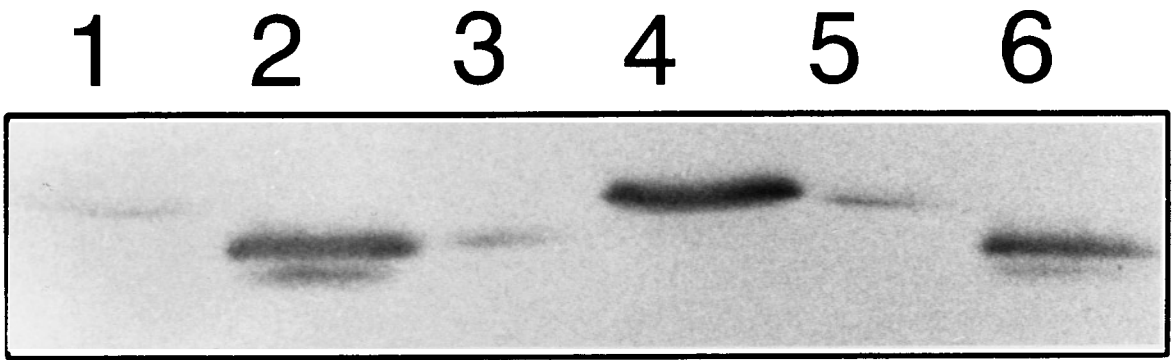

Fig. 3. Expression of HA-α and HA-α′ and derivatives in Cos-7 cells. Cos-7 cells were transfected with pRc/CMV plasmids encoding HA-α (*lane 1*), HA-α′ (*lane 2*), HA-α/α′ (*lane 3*), HA-α′/α (*lane 4*), HA-αk.d. (*lane 5*), and HA-α′k.d. (*lane 6*) in the presence of pRc/CMV encoding myc-β. Lysates of the cells were immunoprecipitated with anti-CK2α (lanes 1, 4, 5) or anti-CK2α′ (lanes 2, 3, 6). The immunoprecipitates were separated by sodium dodecyl sulfate gel electrophoresis, transferred to a nitrocellulose membrane, and then probed with 12CA5 monoclonal antibody to detect the HA-tagged proteins. Immune complexes were visualized using enhanced chemiluminescence.

those observed with HA-α and its derivatives (compare Fig. 4A with Fig. 4B). These results are consistent with the dramatically lower levels of HA-α and its derivatives observed on immunoblots (Fig. 3). Importantly, these results demonstrate that HA-α and HA-α′ exhibit kinase activity.

Coexpression of myc-β with HA-α or HA-α′ results in activity that is increased over that observed in the absence of myc-β, suggesting that complexes between HA-α (or HA-α′) and myc-β had formed. To examine this possibility, we analyzed immunoprecipitates prepared with 12CA5 (anti-HA) monoclonal antibodies on immunoblots for the presence of myc-β (Fig. 5). Since myc-β does not react with 12CA5 antibodies, the presence of myc-β in these immunoprecipitates results from its association with the various forms of HA-α′. Coexpression of HA-α′ with myc-β alone results in higher expression of HA-α′ than expression of HA-α′ alone (Fig. 5, lanes 1, 2). Also, myc-β coprecipitates with the HA-α′ in 12CA5 immunoprecipitates, indicating that HA-α′ is able to associate with myc-β. Two distinct bands were detected with 9E10 (anti-myc) antibodies, the upper one representing the autophosphorylated protein and the lower one representing a nonphosphorylated form [Litchfield et al., 1990, 1991; Luscher and Litchfield, 1994]. Both HA-α′/α and HA-α′k.d. (Fig. 5, lanes 3 and 4, respectively) also show association with myc-β. In the presence of HA-α′ or HA-α′/α, most of the myc-β exhibits reduced mobility characteristic of the autophosphorylated protein. By comparison, in the presence of the kinase-inactive variant of α′ (HA-α′k.d.) myc-β displays the characteristics of the nonphosphorylated protein. Immunoprecipitations are not performed under conditions that would permit phosphorylation so the phosphorylation state would be the result of intracellular events. One explanation for these results is that the autophosphorylation of CK2β in cells occurs through an intramolecular process. On longer exposures, a small amount of autophosphorylated myc-β was observed in immunoprecipitates of HA-α′k.d. (data not shown). This low level of autophosphorylation could result from complexes of HA-α′k.d. that contain endogenous CK2α or CK2α′. These results are consistent with the low levels of kinase observed in 12CA5 immunoprecipitates prepared from cells transfected with HA-α′k.d. Myc-β is not observed when cells are transfected only with HA-α′ (Fig. 5, lane 1) or with myc-β in the presence of an empty vector (pRc/CMV lacking insert, Fig. 5, lane 5). Immunoprecipitations from cells transfected with HA-α and myc-β also suggest that HA-α is capable of forming complexes with CK2β (data not shown). Importantly, these results demonstrate that the HA epitope on CK2α or CK2α′ and the myc epitope on CK2β do not obviously influence interactions between the CK2 subunits.

Subcellular Localization of HA-α and HA-α′

To examine the subcellular distribution of the two isozymic forms of CK2 in transiently transfected Cos-7 cells, HA-α or HA-α′ was visualized by indirect immunofluorescence using 12CA5 antibodies as the primary antibody and FITC-conjugated antimouse antibodies as

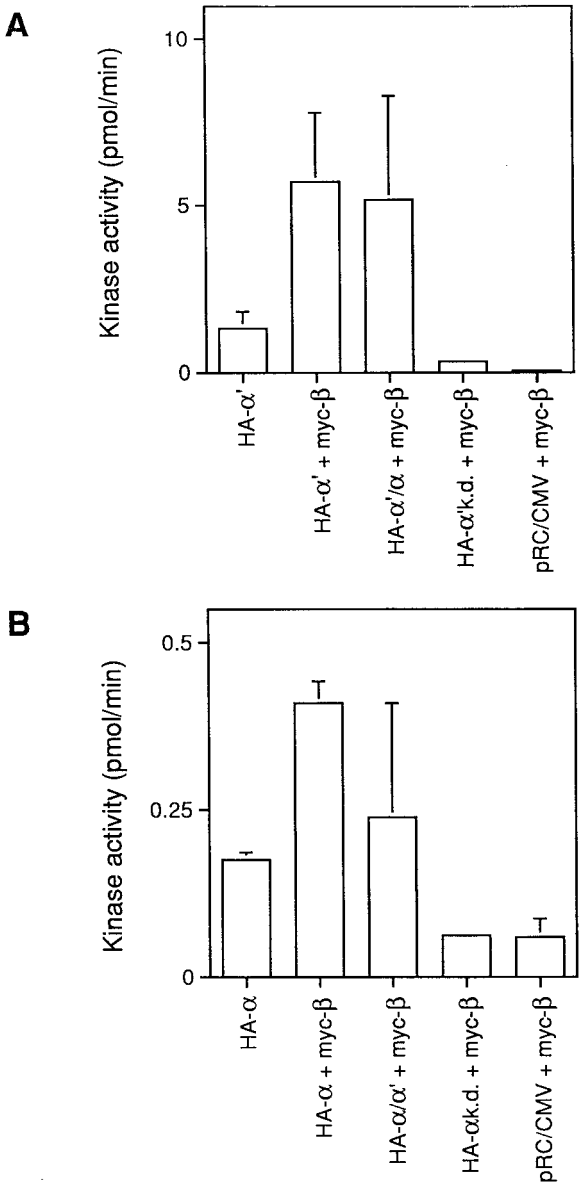

Fig. 4. Kinase activity of HA-α and HA-α′ and derivatives. Cell lysates were prepared from cells that had been transfected with the indicated constructs. Immunoprecipitates were performed using 12CA5 antibodies and immune-complex kinase assays performed as described in Materials and Methods. Kinase activities are expressed in picomoles/minute. The results shown are from two independent experiments (range is indicated by error bars). **A:** Cos-7 cells were transfected with HA-α′, HA-α′ and myc-β, HA-α′/α and myc-β, HA-α′k.d. and myc-β, or pRc/CMV and myc-β. **B:** Cos-7 cells were transfected with HA-α, HA-α and myc-β, HA-α/α′ and myc-β, HA-αk.d. and myc-β, or pRc/CMV and myc-β.

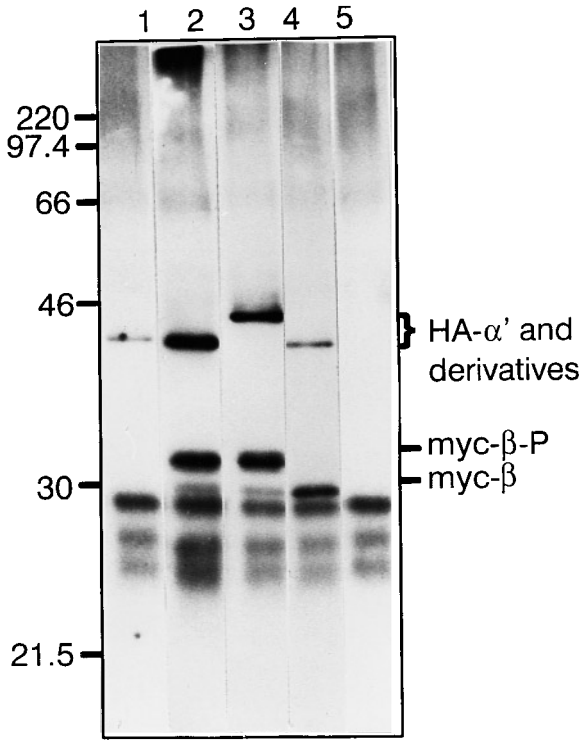

Fig. 5. Formation of complexes between HA-α′ and myc-β. Cos-7 cells were transfected with HA-α′ (*lane 1*), HA-α′ and myc-β (*lane 2*), HA-α′/α and myc-β (*lane 3*), HA-α′k.d. and myc-β (*lane 4*), or pRc/CMV (empty vector) and myc-β (*lane 5*). Cell lysates were immunoprecipitated with 12CA5 antibody as described in Materials and Methods. Immunoprecipitates were analyzed on immunoblots using 12CA5 and 9E10 monoclonal antibodies to detect HA-α′ or its derivatives and myc-β, respectively. Immunoreactivity was visualized by enhanced chemiluminescence. The three bands exhibiting apparent molecular weights less than that of either of the myc-β bands are immunoglobulin bands. These bands are evident in all lanes, including those derived from cells that were not transfected with myc-β.

the secondary antibody (Fig. 6A,C). Endogenous CK2α or CK2α′ were detected on the same slide as HA-α or HA-α′ using anti-α antiserum or affinity-purified anti-α′ antibodies as primary antibodies and TRITC-conjugated goat antirabbit antibodies as the secondary antibodies (Fig. 6B,D). The same fields were photographed with either the FITC or the TRITC fluorescence visible. Endogenous CK2α (Fig. 6B) and HA-α (Fig. 6A) are both localized primarily to the nucleus. Since the cells that have been analyzed are a population of cells that have been transiently transfected and represent a mixture of transfected and untransfected cells, the HA-tagged proteins are not visible in all cells. For example, while a total of six cells is visible when staining with anti-CK2α antiserum which detects endogenous and recombinant protein, only two cells are visible in the same field when staining for HA-α. Endogenous CK2α′ was also localized predominantly to the nucleus (Fig. 6D) as was HA-α′ (Fig. 6C). Most cells transfected with HA-α′ also showed much

brighter staining with anti-CK2α′ antibodies (Fig. 6D) than did untransfected cells, presumably because the overall cellular levels of CK2α′ had been dramatically increased. By comparison, cells that had been transfected with HA-α did not yield such a dramatic increase in staining with anti-CK2α antisera, presumably because HA-α was expressed to lower levels than HA-α′ (Figs. 3, 4). In cells transfected with HA-CK2α′ and with longer exposures of staining for CK2α, CK2α′, and HA-α, some cytoplasmic staining was also observed, indicating that these proteins are not localized exclusively to the nucleus.

For immunofluorescence experiments, a number of controls were performed. The $\alpha^{376-391}$ peptide (Fig. 2A) that was used to raise antibodies against CK2α competes out the immunofluorescent signal detected using anti-CK2α antibodies (data not shown). Use of the antigenic $\alpha^{376-391}$ peptide does not affect immunofluorescent detection of HA-α or any of its derivatives using 12CA5 antibodies (data not shown). In a similar vein, incubation of anti-CK2α′ antibodies with antigenic $\alpha'^{333-350}$ peptide which competes out the signal on immunoblots (Fig. 2B) significantly diminished the immunofluorescent signal (data not shown). By comparison, when the same population of transfected cells was analyzed using 12CA5 antibodies to detect the HA-tagged protein, nuclear staining was not affected by the presence of the $\alpha'^{333-350}$ peptide. Additional controls, including the use of normal rabbit serum or purified mouse immunoglobulin as primary antibodies or the use of secondary antibodies (i.e., TRITC-conjugated

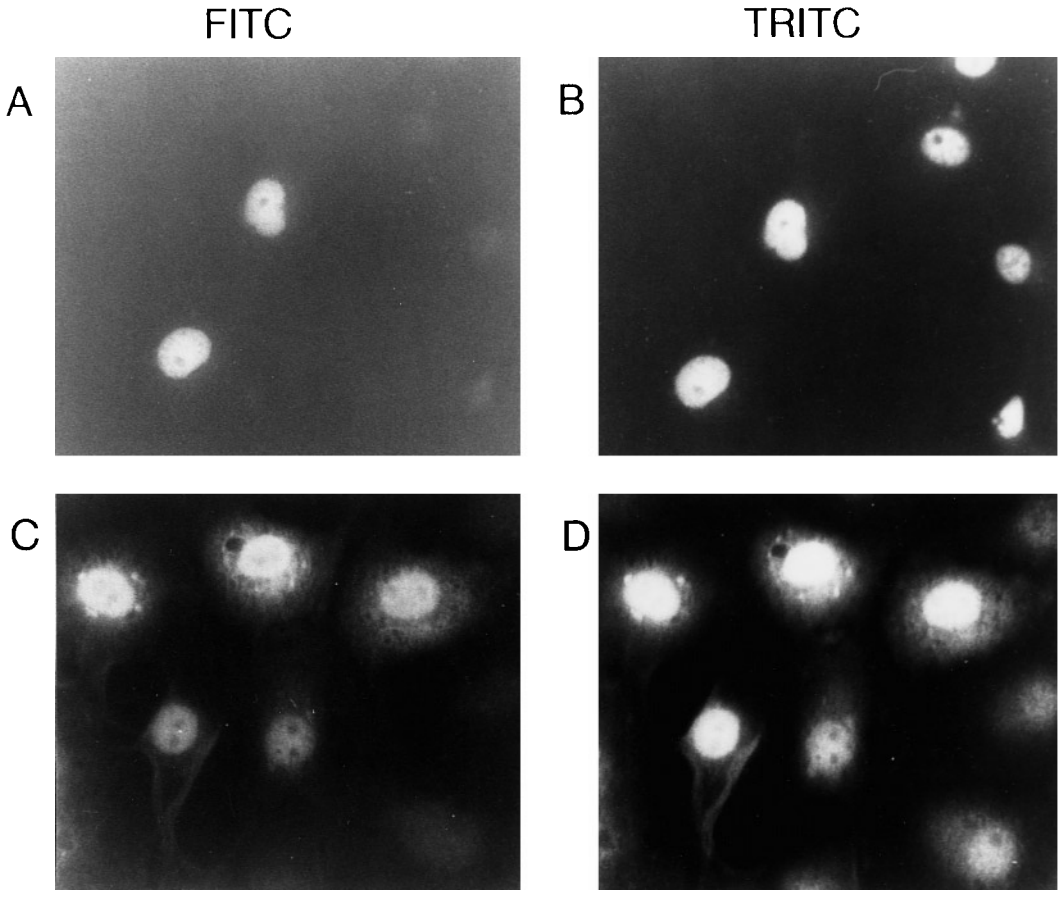

Fig. 6. Indirect immunofluorescent detection of expressed HA-α and HA-α′ and endogenous CK2α and CK2α′. Cos-7 cells were transfected with pRc/CMV encoding HA-α (**A,B**) or with pRc/CMV encoding HA-α′ (**C,D**). The cells were fixed and stained with 12CA5 antibodies and anti-CK2α (A,B) or with 12CA5 antibodies and anti-CK2α′ (C,D) as described in Materials and Methods. The 12CA5 monoclonal antibodies were detected using FITC-conjugated goat antimouse antibodies (A,C), while anti-CK2α (B) and anti-CK2α′ (D) were detected using TRITC-conjugated goat antirabbit antibodies. The same fields from each transfection were photographed for FITC staining and for TRITC staining.

goat antirabbit antibodies or FITC-conjugated goat antimouse antibodies) in the absence of primary antibodies, all failed to yield a significant immunofluorescent signal (data not shown). Furthermore, 12CA5 antibodies failed to produce a signal when used to analyze nontransfected cells or cells that were transfected with pRc/CMV as an empty vector control (data not shown).

To determine whether kinase activity is required for the appropriate subcellular localization of CK2α and CK2α′, we performed indirect immunfluorescence studies on cells that had been transfected with HA-CK2αk.d. or with HA-CK2α′k.d (Fig. 7). Examination of the immunofluorescent signal resulting from staining with 12CA5 antibodies demonstrates that HA-αk.d. is predominantly localized to the nucleus (Fig. 7A). Staining with anti-CK2α also reveals predominant nuclear staining as in previous experiments. Similar observations are obtained by examination of cells transfected with HA-α′k.d., revealing that this recombinant protein is also localized primarily to the nucleus. As we had previously observed with cells transfected with HA-α′, those cells transfected with HA-α′k.d. stain much more brightly than nontransfected cells when using anti-CK2α′ antibodies. Once again, these results suggest that the overall levels of CK2α′ have been dramatically increased in the transfected cells. Overall, the subcellular distribution of HA-αk.d. is very similar to that of HA-α, and the subcellular distribution of HA-α′k.d. is very similar to that of HA-α′. These results demonstrate that catalytic function is not essential for the localization of CK2α or CK2α′ to the nucleus.

DISCUSSION

In mammals and birds, two distinct isozymic forms of the catalytic subunit of CK2, CK2α and CK2α′, have been identified [Lozeman et

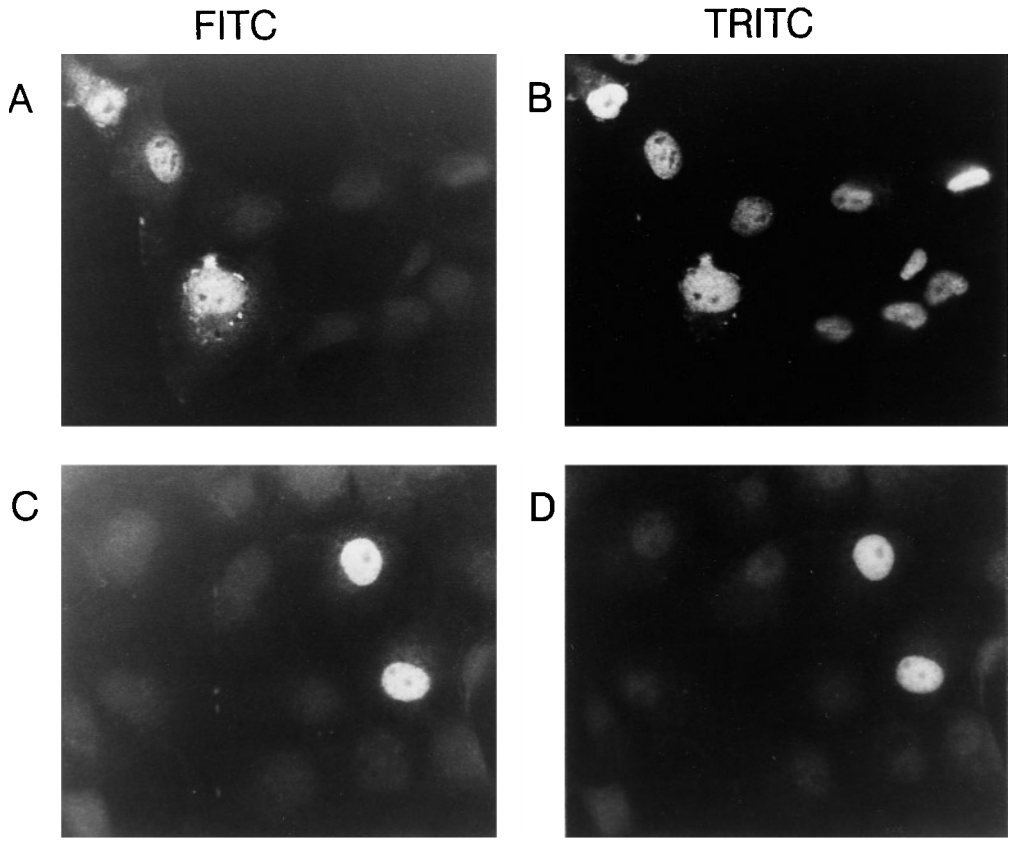

Fig. 7. Indirect immunofluorescent detection of HA-αk.d. and HA-α′kd. Cos-7 cells were transfected with HA-αk.d. (**A,B**) or HA-α′k.d. (**C,D**). The cells were fixed and stained with 12CA5 antibodies and either anti-CK2α (A,B) or with 12CA5 antibodies and anti-CK2α′ (C,D) as described in Materials and Methods. The 12CA5 monoclonal antibodies were detected using FITC-conjugated goat antimouse antibodies (A,C), while anti-CK2α (B) and anti-CK2α′ (D) were detected using TRITC-conjugated goat antirabbit antibodies. The same fields were photographed from each transfection for FITC staining and for TRITC staining.

al., 1990; Maridor et al., 1991]. Between humans and chickens, each of the isozymes exhibits remarkable conservation, suggesting that, since the unique features of each isozyme are conserved, they may have unique functions. In this regard, studies performed by Yu et al. [1991] demonstrated dramatic differences in the subcellular localization of CK2α and CK2α′. However, these conclusions were not supported by a subsequent study by Krek et al. [1992], where considerable overlap between the subcellular localization of the two isozymes was observed. Since CK2α and CK2α′ share extensive sequence identity, many of the conclusions of Yu et al. [1991] were based on analyses using antibodies that exhibit reactivity with both isozymes. In addition to concerns of antibody cross-reactivity, CK2 is known to exist in homotetrameric ($\alpha_2\beta_2$, $\alpha'_2\beta_2$) and in heterotetrameric ($\alpha\alpha'\beta 2$) complexes. Therefore, unless the composition of these complexes is known, it is not possible to draw definitive conclusions regarding the localization of CK2α or CK2α′. To circumvent these problems and to definitively resolve discrepancies regarding the subcellular localization of CK2α and CK2α′, we have examined the subcellular distribution of epitope-tagged variants of the two isozymes. The HA epitope facilitates detection of expressed proteins above a background of endogenous CK2 without concerns of cross-reactivity with other CK2 isoforms. Furthermore, since transfected cells overexpress only one CK2 isoform which can be specifically detected by the antiepitope antibodies, our results are not confounded by the existence of tetrameric complexes containing both CK2α and CK2α′. Overall, our results support the conclusion that both CK2α and CK2α′ are predominantly localized to the nucleus.

The observation that both isoforms of CK2 are primarily localized within the nucleus is consistent with the involvement of CK2 in the phosphorylation of a number of regulatory nuclear proteins [reviewed in Issinger, 1993; Litchfield and Luscher, 1993; Allende and Allende, 1995]. Although the nuclear staining is most prominent with both CK2α and CK2α′, there are indications that a small proportion of each isozyme is also cytoplasmic. The latter observations are consistent with the role of CK2 in the phosphorylation of cytosolic proteins. Furthermore, both CK2α and CK2α′ have been identified in platelets [Hoyt et al., 1994], a model system that is devoid of nuclei.

It is not yet clear to what extent CK2 isoforms are redundant or to what extent they exhibit specialization. We have previously demonstrated that CK2α is phosphorylated in mitotic cells at sites within its carboxy-terminal domain [Litchfield et al., 1992; Bosc et al., 1995]. By comparison, CK2α′ has an unrelated carboxy-terminal domain and is not phosphorylated in mitotic cells. These results suggest that certain functional properties of the two isoforms of CK2 are independently regulated at different stages within the cell cycle. The fact that all mitotic phosphorylation sites are conserved in the deduced sequence of chicken CK2α is consistent with the notion that phosphorylation plays a role in regulation of CK2. The role of phosphorylation in regulating the functions of CK2α is currently under investigation. Differences in the levels of mRNA or protein expression have been noted for CK2α and CK2α′ [Maridor et al., 1991; Krek et al., 1992]. However, these studies do not yield any specific insights into possible unique functions of either of the isoforms. In the yeast *Saccharomyces cerevisiae*, there are also two distinct isozymic forms of CK2 [Chen-Wu et al., 1988; Padmanabha et al., 1990]. Viability is not obviously compromised when the gene encoding only one of the isozymes is disrupted. However, there are indications that loss of one isoform results in morphological alterations, while loss of the other isoform affects cell cycle progression [Hanna et al., 1995]. A precise understanding of the functions of the two isozymic forms of CK2 in mammalian cells must await isolation, or creation, of model systems lacking either of the isoforms.

In vitro, the β subunit of CK2 is known to undergo autophosphorylation in an intramolecular process [reviewed in Pinna, 1990]. In a previous study, we demonstrated that the autophosphorylation site is phosphorylated in cells [Litchfield et al., 1991]. In the present study, we now present evidence that suggests that the autophosphorylation of CK2 also occurs through an intramolecular process in cells. In vitro studies with mutants lacking the autophosphorylation site(s) suggest that autophosphorylation modulates the activity of CK2 towards some but not all substrates [Lin et al., 1994]. The effects of such mutations on the functions of CK2 in cells have not yet been examined. Muta-

tions of the autophosphorylation site do not effect the ability of CK2β to form complexes with CK2α when recombinant subunits are reconstituted in vitro [Meggio et al., 1993; Lin et al., 1994]. In a similar vein, our data now suggest that neither kinase activity nor autophosphorylation of CK2β is required to form stable complexes between CK2β and the catalytic subunits of CK2. It is also apparent that less HA-α′k.d. is obtained in immunoprecipitates than HA-α′ when either of these proteins is coexpressed with myc-β (Fig. 5). We do not know the precise mechanism responsible for this finding. However, it may be important to note that the highest levels of HA-α′ expression are achieved under those circumstances where its catalytic activity is highest (i.e., when expressed in the presence of myc-β). Lesser levels of expression are observed when lesser levels of catalytic activity are present (i.e., with HA-α′k.d. or when HA-α′ is expressed in the absence of myc-β). Therefore, these results may suggest that catalytic activity plays a role in controlling the stability or steady-state levels of CK2.

Our experiments indicate that the HA epitope does not prevent the formation of complexes between expressed CK2α (or CK2α′) and CK2β subunits or the expression of kinase activity. Furthermore, kinase activity is not required for the formation of complexes between the catalytic subunits of CK2 and CK2β, nor is kinase activity necessary for localization of CK2 to the nucleus. At present the factors that control the transport of CK2 to the nucleus have not been defined. Interestingly, Chester et al. [1995] suggested from biosynthetic labelling experiments that the nucleus was a major site of assembly for CK2α, CK2α′, and CK2β complexes, suggesting that the individual subunits of CK2 enter the nucleus prior to holoenzyme formation. Since both CK2α and CK2α′ contain sequences that conform to known nuclear localization signals, the ability of both isoforms to localize to the nucleus may reside within these sequences. By comparison, since CK2β does not contain an obvious nuclear localization signal, the mechanisms by which CK2β is transported to the nucleus remains poorly understood.

The results of the present study suggest that the kinase-inactive mutants of CK2α and CK2α′ may behave as dominant-negative mutants. Consequently, it will be of interest to determine whether overexpression of kinase-inactive CK2α or CK2α′ can affect specific signalling events in which CK2 is involved. In this way it may be possible to define the unique functions of CK2α and CK2α′.

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REFERENCES

Allende JE, Allende CC (1995): Protein kinase CK2: An enzyme with multiple substrates and a puzzling regulation. FASEB J 9:313–323.

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl KA (1990): "Current Protocols in Molecular Biology." Boston: Wiley-Interscience.

Birnbaum MJ, Glover CVC (1991): The phosphotransferase activity of casein kinase II is required for its physiological function in vivo. Biochem Biophys Res Commun 181:524–528.

Bodenbach L, Fauss J, Robitzki A, Krehan A, Lorenz P, Lozeman FJ, Pyerin W (1994): Recombinant casein kinase II. Eur J Biochem 220:263–273.

Boldyreff B, Meggio F, Pinna LA, Issinger OG (1993): Reconstitution of normal and hyperactivated forms of casein kinase-2 by variably mutated beta-subunits. Biochemistry 32:12672–12677.

Bosc DG, Slominski E, Sichler C, Litchfield DW (1995): Phosphorylation of casein kinase II by p34cdc2: Identification of phosphorylation sites using phosphorylation site mutants in vitro. J Biol Chem 270:25872–25878.

Chadee DN, Taylor WR, Hurta RA, Allis CD, Wright JA, Davie JR (1995): Increased phosphorylation of histone H1 in mouse fibroblasts transformed with oncogenes or constitutively active mitogen-activated protein kinase kinase. J Biol Chem 270:20098–20105.

Chen Wu JL, Padmanabha R, Glover CV (1988): Isolation, sequencing, and disruption of the CKA1 gene encoding the alpha subunit of yeast casein kinase II. Mol Cell Biol 8:4981–4990.

Chester N, Yu IJ, Marshak DR (1995): Identification and characterization of protein kinase CKII isoforms in Hela cells. J Biol Chem 270:7501–7514.

Egan SE, Giddings BW, Brooks MW, Buday L, Sizeland AM, Weinberg RA (1993): Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine signal transduction and transformation. Nature 363:45–51.

Evan GI, Lewis G, Ramsey G, Bishop JM (1985): Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. Mol Cell Biol 5:3610–3616.

Field J, Nikawa J-I, Broek D, Macdonald B, Rodgers I, Wilson IA, Lerner RA, Wigler M (1988): Purification of RAS-responsive adenylyl cyclase complex form *S. Cerevi-*

siae by use of an epitope addition method. Mol Cell Biol 8:2159–2165.

Filhol O, Cochet C, Wedegaertner P, Gill GN, Chambaz EM (1991): Coexpression of both alpha and beta subunits is required for assembly of regulated casein kinase II. Biochemistry 30:11133–11140.

Gietz RD, Graham KC, Litchfield DW (1995): Interactions between the subunits of casein kinase II. J Biol Chem 270:13017–13021.

Hanks SK, Quinn AM (1991): Protein kinase catalytic domain sequence database: Identification of conserved features of primary structure and classification of family members. Methods Enzymol 200:38–62.

Hanna DE, Rethinaswamy A, Glover CVC (1995): Casein kinase II is required for cell cycle progression during G1 and G2/M in *Saccharomyces cerevisiae*. J Biol Chem 270:25905–25914.

Harlow E, Lane D (1988): "Antibodies: A Laboratory Manual." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Heller-Harrison RA, Czech MP (1991): Enhanced casein kinase II activity in COS-1 cells upon overexpression of either its catalytic or noncatalytic subunit. J Biol Chem 266:14435–14439.

Hoyt CH, Oh CJ, Beekman JB, Litchfield DW, Lerea KM (1994): Identifying and characterizing casein kinase II in human platelets. Blood 83:3517–3523.

Hu E, Rubin CS (1990): Expression of wild-type and mutant forms of the catalytic subunit of *Caenorhabditis elegans* casein kinase II in *Escherichia coli*. J Biol Chem 265:20609–20615.

Innis MA, Gelfand DH, Sninsky JJ, White TJ (1990): "PCR Protocols: A Guide to Method and Applications." San Diego: Academic Press.

Issinger OG (1993): Casein kinases: pleiotropic mediators of cellular regulation. Pharmacology and Therapeutics. 59:1–30.

Kikkawa U, Mann SK, Firtel RA, Hunter T (1992): Molecular cloning of casein kinase II alpha subunit from *Dictyostelium discoideum* and its expression in the life cycle. Mol Cell Biol 12:5711–5723.

Krek W, Maridor G, Nigg EA (1992): Casein kinase II is a predominantly nuclear enzyme. J Cell Biol 116:43–55.

Laemmli UK (1970): Cleavage of the structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Lin WJ, Tuazon PT, Traugh JA (1991): Characterization of the catalytic subunit of casein kinase II expressed in *Escherichia coli* and regulation of activity. J Biol Chem 266:5664–5669.

Lin WJ, Sheu GT, Traugh JA (1994): Effects of autophosphorylation on casein kinase II activity: Evidence from mutations in the beta subunit. Biochemistry 33:86–80.

Litchfield DW, Luscher B (1993): Casein kinase II in signal transduction and cell cycle regulation. Mol Cell Biochem 127–128:187–199.

Litchfield DW, Lozeman FJ, Piening C, Sommercorn J, Takio K, Walsh KA, Krebs EG (1990): Subunit structure of casein kinase II from bovine testis. Demonstration that the alpha and alpha′ subunits are distinct polypeptides. J Biol Chem 265:7638–7644.

Litchfield DW, Lozeman FJ, Cicirelli MF, Harrylock M, Ericsson LH, Piening CJ, Krebs EG (1991): Phosphorylation of the beta subunit of casein kinase II in human A431 cells. Identification of the autophosphorylation site and a site phosphorylated by p34cdc2. J Biol Chem 266: 20380–20389.

Litchfield DW, Luscher B, Lozeman FJ, Eisenman RN, Krebs EG (1992): Phosphorylation of casein kinase II by p34cdc2 in vitro and at mitosis. J Biol Chem 267:13943–13951.

Lozeman FJ, Litchfield DW, Piening C, Takio K, Walsh KA, Krebs EG (1990): Isolation and characterization of human cDNA clones encoding the alpha and the alpha′ subunits of casein kinase II. Biochemistry 29:8436–8447.

Luscher B, Litchfield DW (1994): Biosynthesis of casein kinase II in lymphoid cell lines. Eur J Biochem 220:521–526.

Maridor G, Park W, Krek W, Nigg EA (1991): Casein kinase II. cDNA sequences, developmental expression, and tissue distribution of mRNAs for alpha, alpha′, and beta subunits of the chicken enzyme. J Biol Chem 266:2362–2368.

Meggio F, Boldyreff B, Issinger OG, Pinna LA (1993): The autophosphorylation and p34cdc2 phosphorylation sites of casein kinase-2 beta-subunit are not essential for reconstituting the fully-active heterotetrameric holoenzyme. Biochim Biophys Acta 1164:223–225.

Padmanabha R, Chen-Wu JL, Hanna DE, Glover CV (1990): Isolation, sequencing, and disruption of the yeast CKA2 gene: Casein kinase II is essential for viability in *Saccharomyces cerevisiae*. Mol Cell Biol 10:4089–4099.

Pinna LA (1990): Casein kinase 2: An 'eminence grise' in cellular regulation? Biochim Biophys Acta 1054:267–284.

Roussou I, Draetta G (1994): The *Schizosaccharomyces pombe* casein kinase II alpha and beta subunits: Evolutionary conservation and positive role of the beta subunit. Mol Cell Biol 14:576–586.

Sambrook J, Fritsch EF, Maniatis T (1989): "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sanger F, Nicklen S, Coulson AR (1977): DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci U S A 74:5463–5467.

Seldin DC, Leder P (1995): Casein kinase II alpha transgene-induced murine lymphoma: Relation to theileriosis in cattle. Science 267:894–897.

Snell V, Nurse P (1994): Genetic analysis of cell morphogenesis in fission yeast— a role for casein kinase II in the establishment of cell growth. EMBO J 13:2066–2074.

Towbin H, Staehelin T, Gordon J (1979): Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci U S A 76:4350–4354.

Tyers M, Tokiwa G, Nash R, Futcher B (1992): The Cln3-Cdc28 kinase complex of *S. cerevisiae* is regulated by proteolysis and phosphorylation. EMBO J 11:1773–1784.

Wilson IA, Niman HL, Houghten RA, Cherenson AR, Connolly ML, Lerner RA (1984): The structure of an antigenic determinant in a protein. Cell 37:767–778.

Yu IJ, Spector DL, Bae YS, Marshak DR (1991): Immunocytochemical localization of casein kinase II during interphase and mitosis. J Cell Biol 114:1217–1232.