The Regulatory β Subunit of Protein Kinase CK2 Mediates Formation of Tetrameric CK2 Complexes*

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Protein kinase CK2 is a tetrameric enzyme composed of two catalytic (α and/or α') subunits and two regulatory (β) subunits. Because CK2β is synthesized in excess of CK2α, we hypothesized that formation of CK2β homodimers precedes the incorporation of the catalytic subunits of CK2 into complexes. To test this hypothesis, we cotransfected cells with two epitope-tagged variants of CK2β. The results of these cotransfection studies demonstrate that interactions between two CK2β subunits take place in the absence of CK2α. Together with results from previous biosynthetic labeling studies, these results suggest that formation of CK2β homodimers occurs before incorporation of catalytic subunits of CK2 into CK2 complexes. We also cotransfected Cos-7 cells with a deletion fragment of CK2β (i.e. Myc-β1–166) together with full-length hemagglutinin (HA)-tagged CK2β and/or CK2α. Although complexes between Myc-β1–166 and HA-β were readily detected, we obtained no evidence of direct interactions between Myc-β1–166 and HA-CK2α. These results suggest that residues within the N-terminal 166 amino acids of CK2β are sufficient for interactions between CK2β subunits, whereas the C-terminal domain of CK2β is required for complex formation with the catalytic subunits of CK2. Finally, we observed that expression of full-length HA-β promotes phosphorylation of Myc-β1–166 by HA-CK2α.

Protein kinase CK2 (formerly known as casein kinase II) is a protein serine/threonine kinase involved in various aspects of cellular regulation (1–4). The enzyme is ubiquitously distributed in eukaryotic organisms and is essential for viability (5–7). There have been a number of reports that CK2 is overexpressed in tumors or in leukemic cells (8–11). Furthermore, the dysregulated expression of CK2 in the lymphocytes of transgenic mice results in lymphocyte transformation. In these transgenic mice, CK2 exhibited cooperation with c-Myc or with c-erbB in transformation (12, 13). Collectively, these results suggest that CK2 is a regulatory component of the protein kinase networks that regulate the growth and division of cells.

There is a significant body of evidence demonstrating that CK2 phosphorylates and regulates the activity of numerous proteins involved in the control of various aspects of cellular function (1–4). However, the regulation of CK2 in intact cells remains an area of considerable controversy (14). In general, there is a consensus that the majority of CK2 in cells exists as a tetrameric complex composed of two catalytic (designated α and/or α') and two noncatalytic β subunits (1–4). The catalytic subunits contain all of the conserved consensus motifs for members of the protein kinase family. By comparison, in mammals, the β subunit of CK2 does not share extensive homology with any known proteins. It is noteworthy that CK2β exhibits remarkable conservation between species. In fact, the deduced amino acid sequences of human and chicken CK2β are identical. The deduced sequences of Xenopus CK2β and zebrafish CK2β differ from those of human and chicken CK2β by only one and two amino acids, respectively (4). This high level of evolutionary conservation suggests that CK2β has important cellular functions. By itself, CK2β has no known catalytic activity, but it appears to regulate the activity of CK2α in a number of ways. In particular, CK2β stabilizes CK2α and modulates the ability of CK2α to interact with and phosphorylate substrate proteins (15–18). Furthermore, CK2β appears to mediate the activating effects of compounds such as polyamines that may have a role in regulating CK2 in cells (19, 20). Overall, these results suggest that CK2β is a critical mediator of the cellular functions of CK2.

To fully define the cellular functions of CK2 and to understand its regulation, a thorough understanding of the functions of each of its subunits is critical. In recent studies using the yeast two-hybrid system, we and others demonstrated that CK2β has the ability to interact with CK2α or with CK2α', whereas CK2α is only able to interact with CK2β (21–23). These results suggested that interactions between two β subunits are responsible for bringing two αβ dimers into active tetrameric complexes. However, because yeast contain endogenous CK2, it was not possible to exclude the possibility that indirect interactions between two CK2β subunits were being mediated by endogenous CK2α. From biosynthetic labeling studies, we had also demonstrated that CK2β is synthesized in excess of CK2α (24). Furthermore, these studies demonstrated that newly synthesized CK2α is rapidly incorporated into complexes with CK2β, whereas newly synthesized CK2β associates more slowly into complexes with CK2α. Consequently, we hypothesized that formation of complexes between CK2β subunits precedes the incorporation of catalytic subunits into tetrameric complexes. In the present study, we provide evidence from transfection studies using two epitope-tagged versions of CK2β (i.e. CK2β with an N-terminal HA epitope designated HA-CK2β and CK2β with an N-terminal Myc epitope designated Myc-CK2β) to support this hypothesis. Furthermore, toward the objective of controlling the activity of CK2 within cells by controlling its ability to form tetrameric complexes, we

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The abbreviations used are: CK2, protein kinase CK2 or casein kinase II; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis; CMV, cytomegalovirus.

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have also examined the ability of individual domains of CK2β to interact with full-length CK2α and/or CK2β in cells. In this regard, we have demonstrated that an N-terminal fragment of CK2β encoding residues 1–166 retains the ability to interact with CK2β in cells but fails to directly form stable complexes with CK2α. These results indicate that residues required for stable interactions between two CK2β subunits in cells are localized within the N-terminal 166 residues of CK2β. By comparison, stable interactions between CK2β and the catalytic subunits of CK2 require additional residues. Importantly, our results suggest that deletion mutants of CK2β such as CK2β1–166 offer new strategies for controlling the functions of CK2 in cells by altering the subunit composition of CK2 in cells in a manner that prevents formation of intact tetrameric CK2 complexes.

MATERIALS AND METHODS

Antibodies—Antipeptide antibodies directed against CK2α (anti-CK2α333–350), against CK2α* (anti-CK2α333–350), and against CK2β (anti-CK2β396–215) have been described previously (25, 26, 36). Purified monoclonal 12CA5 antibodies directed against the HA epitope (27) were purchased from Berkeley Antibody Company (Richmond, CA). Biotinylated rat monoclonal anti-HA antibodies (clone 3F10) were obtained from Roche Molecular Biochemicals, and peroxidase-conjugated mouse monoclonal anti-biotin antibodies were obtained from Jackson ImmunoResearch Laboratories Inc. The hyridoma producing 9E10 monoclonal antibodies directed against the Myc epitope (28) were obtained from the American Type Culture Collection (Manassas, VA). Cells were used to produce ascites fluid in mice, and antibodies were partially purified from ascites fluid by ammonium sulfate precipitation.

Plasmids Constructs—Full-length HA-CK2α and Myc-CK2β, containing N-terminal HA or Myc epitope tags, respectively, were expressed using pRC/CMV as described previously (18). Full-length CK2β containing an N-terminal HA tag was constructed using a strategy similar to that used for construction of HA-CK2α. Briefly, a Ndel site was introduced at the 5′ end of the coding region of the CK2β cDNA using the polymerase chain reaction with the following primers: sense (ATA AGA ATG CGG CCG CAG CAG CTC AGA GGA G) and antisense (CTT GGG GCA GTA GAG CTT). The amplified sequence was subcloned into the pBluescript II KS(−) plasmid using the Apal site. The construct was verified by sequencing and cloned into pRc/CMV to direct expression of the transfected proteins. Based on this assumption, it appeared that either HA-CK2α or Myc-CK2β were maintained in Dulbecco's modified Eagle's medium (Life Technologies). Transfection of mammalian cells using the CMV promoter to direct expression of the transfected constructs or with secondary antibodies conjugated to alkaline phosphatase for color development with bromochloroindolyl phosphate and nitro blue tetrazolium as substrates. Protein determinations were performed according to the method of Bradford using bovine serum albumin as standard (33).

RESULTS

Complexes between Regulatory CK2 Subunits Form in the Absence of CK2α in Cells—Based on previous observations using the yeast two-hybrid system (21–23) and with support from cross-linking studies (37, 38), it had been demonstrated that the regulatory subunit of CK2 (i.e. CK2β) interacts with CK2α and with CK2β. To determine whether complexes between two CK2β subunits form within cells prior to the formation of complexes with CK2α, we prepared constructs encoding two different epitope-tagged constructs of CK2α, HA-CK2α and Myc-CK2α (Fig. 1A). As demonstrated in Fig. 1, HA-CK2α can be specifically immunoprecipitated with 12CA5 antibodies (B and C, lane 1) and with anti-CK2β396–215 antibodies (B and C, lane 3). Similarly, Myc-CK2α is immunoprecipitated using 9E10 monoclonal antibodies (B and D, lane 5) and with anti-CK2β396–215 (B and D, lane 6). Importantly, the 12CA5 antibodies do not react with Myc-CK2β (B and C, lane 4) and the 9E10 antibodies do not react with HA-CK2β (B and D, lane 2). Endogenous CK2β is also evident in anti-CK2β immunoprecipitates (lanes 3 and 6) and in the anti-Myc immunoprecipitate (lane 5). The presence of endogenous CK2β in anti-Myc immunoprecipitates is indicative of endogenous CK2β in complexes containing Myc-CK2α. The high levels of Myc-CK2β and HA-β synthesis that is observed relative to endogenous CK2β during this 20-min biosynthetic labeling period likely results from the use of the strong CMV promoter to drive the expression of the transfected proteins.

To verify that HA-CK2β and Myc-CK2β are functional with respect to their capacity to interact with CK2α, we demonstrated that either HA-CK2β (Fig. 2B) or Myc-CK2β (Fig. 2C) can be detected in specific anti-CK2α379–391 immunoprecipitates. Moreover, we also demonstrated that the HA-CK2β and Myc-CK2β signals are specific to complexes containing endogenous CK2α by performing immunoprecipitations in the presence of an anti-CK2α376–391 peptide (Fig. 2, A–C). As previously noted (18), the HA-CK2β or Myc-CK2β bands of different electrophoretic mobility most likely reflect different phosphorylated forms of the proteins. Based on this assumption, it ap-
lates that a significant proportion of HA-CK2β and Myc-CK2β are phosphorylated in these complexes that are isolated by immunoprecipitation with anti-CK2α antibodies. The observation that kinase activity toward a CK2-specific synthetic peptide substrate is detected in the appropriate anti-HA (Fig. 2D) or anti-Myc (Fig. 2E) immunoprecipitates is also indicative of complex formation between the transfected proteins and endogenous catalytic subunits of CK2. We do not have a precise explanation for the significant differences observed for the kinase activities of anti-HA (Fig. 2D) or the anti-Myc (Fig. 2E) immunoprecipitates. However, the differences may in part reflect differences in immunoprecipitation efficiency or perhaps conformational differences between the two antibodies.

Having verified functional expression of HA-CK2β and Myc-CK2β and specificity of isolation and detection, we subsequently cotransfected the two constructs into cells (Fig. 3). We were primarily interested in determining whether the two versions of CK2β are capable of interacting with each other in cells

assembly of Protein Kinase CK2

FIG. 1. Immunoprecipitation of Myc-CK2β and HA-CK2β. A, constructs encoding CK2β with an N-terminal Myc epitope that is reactive with 9E10 monoclonal antibodies (i.e. Myc-β) and with an N-terminal HA epitope that is reactive with 12CA5 monoclonal antibodies (i.e. HA-β) are illustrated. The epitope at the C terminus of CK2β that is recognized by anti-CK2β[355–350] antibodies is also indicated. HA-CK2β is a protein of 254 amino acids including amino acids 2–215 of CK2β. The 40-amino acid tag at the N terminus of this protein contains a triple repeat of the YPYDVPDY epitope sequence derived from influenza virus hemagglutinin that is recognized by 12CA5 monoclonal antibodies. By comparison, Myc-CK2β is a protein of 241 amino acids that includes a 26-amino acid N-terminal sequence with one copy of the EQKISSEEL epitope sequence derived from the c-Myc protein. B, Cos-7 cells were transfected with either pRc/CMV encoding HA-β (lanes 1–3) or Myc-β (lanes 4–6) and then labeled with [35S]methionine/cysteine for 20 min as described under "Materials and Methods." Extracts were prepared and immunoprecipitations performed with 12CA5 antibodies (lanes 1 and 4), with 9E10 antibodies (lanes 2 and 5) or with anti-CK2β[355–350] antibodies (lanes 3 and 6). HA-β and Myc-β, which exhibit similar electrophoretic mobilities, are indicated. Endogenous CK2β is also indicated. C, cell extracts were transfected and immunoprecipitated as in B without radiolabeling. Immunoprecipitates were analyzed on immunoblots with 12CA5 antibodies. D, cell extracts were transfected and immunoprecipitated and analyzed on immunoblots using 9E10 antibodies. The positions of HA-CK2β or Myc-CK2β are indicated in C and D, respectively. The position of the L chain of IgG is also indicated.

FIG. 2. Interaction of HA-CK2β and Myc-CK2β with endogenous CK2α. A, Cos-7 cells were transfected with pRc/CMV encoding HA-CK2β (H), Myc-CK2β (M), or empty (V) vector as indicated. A, cell extracts were immunoprecipitated using anti-CK2α[376–391] antibodies in the presence (+) or absence (−) of 12CA5 antibodies. Immunoprecipitated proteins were separated by SDS-PAGE and analyzed by immunoblotting with anti-CK2α[376–391] antibodies. The position of CK2α is indicated as is the position of the H chain of IgG. B, cell extracts were prepared and immunoprecipitated as in A. Immunoblots were analyzed using 12CA5 antibodies. The nonphosphorylated and phosphorylated forms of HA-β are indicated. C, cell extracts were prepared and immunoprecipitated as in A. Immunoblots were analyzed using 9E10 antibodies. The nonphosphorylated and phosphorylated forms of Myc-β are indicated. D, cell extracts were prepared as in A and immunoprecipitated with 12CA5 antibodies. E, cell extracts were prepared and immunoprecipitates were performed using 9E10 antibodies in preparation for immune complex kinase assays.

FIG. 3. Co-immunoprecipitation of Myc-CK2β and HA-CK2β. A, Cos-7 cells were transfected with pRc/CMV (50 μg/10-cm dish) or with mixtures of pRc/CMV constructs encoding Myc-CK2β and HA-CK2β in the amounts indicated (μg/10-cm dish). Extracts from transfected cells were subjected to two successive rounds of immunoprecipitation with a mixture of anti-CK2α[376–391] and anti-CK2α[333–350] antibodies to deplete extracts of tetrameric CK2 complexes. The depleted extracts were then immunoprecipitated with 9E10 antibodies (first three lanes) or with 12CA5 antibodies (last three lanes) as indicated. Immunoprecipitates were electrophoresed on replicate gels and were then transferred to polyvinylidene difluoride membrane for immunoblotting with 12CA5 (A) or 9E10 (B) antibodies. Bands corresponding to HA-β (A), Myc-β (B), and the L chain of IgG (both panels) are indicated.

in the absence of CK2α. Consequently, two preliminary rounds of immunoprecipitations were carried out using a mixture of anti-CK2α and anti-CK2α′ antibodies to deplete extracts of tetrameric CK2 complexes. These depleted extracts were subsequently immunoprecipitated with either anti-HA or anti-Myc antibodies (Fig. 3). Immunoprecipitations were also performed with the mixture of anti-CK2α and anti-CK2α′ to verify that extracts had indeed been depleted of tetrameric complexes and with anti-CK2β antibodies to verify that the tagged proteins do
formed transfections of HA-CK2 complexes containing catalytic subunits, we next performed transfections of vectors encoding each of the three proteins. Lane 1, empty vector + HA-CK2α + Myc-CK2β; lane 2, empty vector + HA-CK2β + Myc-CK2α; lane 3, HA-CK2α + HA-CK2β + Myc-CK2β; lane 4, empty vector (two equivalents) + Myc-CK2β. Extracts were prepared from transfected cells, and immunoprecipitations were performed with anti-Myc monoclonal antibodies. Immunoprecipitates were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membrane for immunoblotting with 12CA5 antibodies. Immune complexes were detected with enhanced chemiluminescence as described under “Materials and Methods.” The positions of the L chain of IgG is marked as are the positions of HA-CK2α and HA-CK2β (phosphorylated and non-phosphorylated forms).

Indeed, these data suggest that the complexes between Myc-CK2α and HA-CK2β have formed in the absence of a catalytic CK2 subunit.

In a previous study (18), we had demonstrated that HA-CK2α and Myc-CK2β form complexes that can be isolated by co-immunoprecipitation assays. Furthermore, those results indicated that Myc-CK2β is readily autophosphorylated upon formation of complexes with HA-CK2α, whereas in the absence of complex formation, Myc-CK2β remained unphosphorylated (18). Therefore, to demonstrate that complexes between HA-CK2β and Myc-CK2β are capable of forming tetrameric CK2 complexes containing catalytic subunits, we next performed transfections of HA-CK2β and Myc-CK2β in the presence or absence of HA-CK2α (Fig. 4). Myc-CK2β was isolated by immunoprecipitation with 9E10 antibodies, and immunoprecipitates were examined by immunoblots using 12CA5 antibodies (Fig. 4) to detect the presence of co-precipitating HA-CK2α or HA-CK2β. As expected, either HA-CK2α (Fig. 4, lane 1) or HA-CK2β (Fig. 4, lane 2) can be isolated in complexes with Myc-CK2β. Furthermore, both HA-CK2β and HA-CK2α are detected in anti-Myc immunoprecipitates when cells are co-transfected with plasmids encoding Myc-CK2β together with HA-CK2α and HA-CK2β (Fig. 4, lane 3). Importantly, when isolated in the latter complexes, HA-CK2β exhibits a profile similar to that observed in Fig. 2B and appears to be phosphorylated. Because autophosphorylation is an intramolecular process, this result indicates that HA-CK2β is part of a complex that contains HA-CK2α, which provides kinase activity, and with Myc-CK2β, which provides the Myc epitope that is used for isolation of the complex. By comparison, in complexes lacking HA-CK2α (Fig. 4, lane 2), HA-CK2β does not appear to be phosphorylated. In cells that were transfected solely with plasmid encoding Myc-CK2β (Fig. 4, lane 4), neither HA-CK2α nor HA-CK2β are detected. An identical blot probed with anti-Myc confirmed the presence of Myc-CK2β in all lanes (data not shown). Overall, these observations provide further support that the epitope-tagged CK2 subunits are competent for the formation of intact CK2 complexes.

Interactions between CK2β1–166 and CK2α in Transfected Cos-7 Cells—Results from a number of laboratories including our own have led to the elucidation of functional domains of CK2β (15, 19, 22, 23, 38–40). Those results were obtained primarily by in vitro reconstitution of bacterially expressed proteins or through analyses using the yeast two-hybrid system. Overall, those results suggested that CK2β1 forms stable interactions with CK2α through residues within its C-terminal domain and that CK2β interacts with another CK2β subunit through residues with its N-terminal domain. However, there are discrepancies regarding the precise domain(s) of CK2β that are responsible for stable interactions with another CK2β. From the studies of Kusk et al. (23), the region of CK2β that was required for homodimerization was mapped to residues 1–145 of CK2β, whereas the data of Boldyreff et al. (22) suggested that residues 155–165 were required for homodimerization of CK2β. Our own studies with the yeast two-hybrid system were inconclusive (39). To extend the results of our own studies, we were interested in further examining the interaction domains of CK2β in intact mammalian cells. Importantly, we sought to examine these interactions using deletion products with small Myc epitope tags instead of the relatively large fusions that are used in the two-hybrid system. One objective of these studies is to develop strategies for preventing the formation of tetrameric complexes or for altering the subunit composition of CK2 complexes. In initial transfection experiments, we assessed the feasibility of expressing, in Cos-7 cells, a deletion construct of CK2β (designated Myc-CK2β1–166) that contains the elements defined by both Kusk et al. (23) and Boldyreff et al. (22) that are required for interactions between two CK2β subunits (Fig. 5). As seen in Fig. 5, Myc-CK2β1–166 (lane 2) was expressed at a level comparable with that of full-length Myc-CK2β (lane 1). We also transfected cells with constructs encoding
immunoprecipitates of Myc-CK2β1–166 was expressed at levels close to those seen with wild-type CK2β, we cotransfected this construct with either HA-CK2β or with HA-CK2α to examine its ability to form complexes with the other subunits of CK2 in cells. We opted to use HA-CK2α for these experiments rather than HA-CK2α because we had previously observed higher levels of expression with HA-CK2α (18). Furthermore, CK2α and CK2α are very closely related, and there are no obvious indications that they exhibit any differences in their ability to interact with the regulatory CK2β subunit (21). Following cotransfection, Myc-CK2β1–166 was isolated from cell extracts by immunoprecipitation using 9E10 antibodies, and immunoprecipitates were subsequently analyzed for the presence of co-precipitating HA-CK2α or HA-CK2α. As shown in Fig. 6A, HA-CK2α was detected in anti-Myc immunoprecipitates from cells transfected with either Myc-CK2β (lane 4) or Myc-CK2β1–166 (lane 5) but not in immunoprecipitates prepared from cells transfected with HA-CK2β and control vector (lane 6). By comparison, HA-CK2α was readily detected in immunoprecipitates of full-length Myc-CK2β (lane 1) but was not evident in immunoprecipitates of Myc-CK2β1–166 (lane 2) or in immunoprecipitates of cells transfected with HA-CK2α and control vector (lane 3). This result suggests that Myc-CK2β1–166 lacks the elements that are required for the direct formation of stable complexes with the catalytic subunit of CK2 in cells. This result is consistent with the results of the yeast two-hybrid system (23), where a construct encoding the N-terminal 166 residues of CK2β exhibited interactions with CK2β but failed to interact with CK2α.

To further study the formation of CK2 complexes, transfections were performed with Myc-CK2β or Myc-CK2β1–166 and both HA-CK2β and HA-CK2α. Anti-myc immunoprecipitates were performed and analyzed by immunodetection with anti-HA antibodies. To avoid the appearance of the immunoglobulin from immune complexes (as seen in Fig. 6A), these blots were developed using biotinylated anti-HA antibodies that interact with the regulatory CK2β subunit of immune complexes (as seen in Fig. 6). Using these biotinylated antibodies, interactions between HA-CK2α and Myc-CK2β (lane 1) as well as interactions between HA-CK2β and either Myc-CK2β (lane 4) or Myc-CK2β1–166 (lane 5) were confirmed. Again, the majority of the HA-CK2β that exists in complexes with either Myc-CK2β (lane 4) or Myc-CK2β1–166 (lane 5) appears to be unphosphorylated. When cells were transfected with HA-CK2α, and both Myc-CK2β and HA-CK2β, it is likely that tetrameric complexes composed of HA-CK2α/Myc-CK2β/Myc-CK2β/HA-CK2α are formed (lane 7). Consistent with what is observed in lane 7, the ratio of immunoreactive HA-CK2α to immunoreactive HA-CK2β is expected to be 2:1 in these complexes. Furthermore, in accordance with the results of Fig. 4, a significant proportion of the HA-CK2β in these complexes appears to be phosphorylated.

Anti-myc immunoprecipitates were also performed from extracts derived from cells transfected with Myc-CK2β1–166 together with HA-CK2β and HA-CK2α. Analysis of the HA-CK2β in these complexes with Myc-CK2β1–166 (lane 8) illustrates a shift in migration indicative of phosphorylation for much of the HA-CK2β subunit. Overall, the band pattern of the HA-CK2β isolated in complexes with Myc-CK2β1–166 is similar to that seen with complexes containing full-length Myc-CK2β (compare lanes 7 and 8). Because isolated CK2β does not act as a substrate for CK2 (24), we believe that this result may reflect the formation of complexes between Myc-CK2β1–166: HA-CK2β dimers and HA-CK2α. Consistent with this possibility, low levels of HA-CK2α are also detected in these immune complexes (lane 8). The HA-CK2α is more clearly evident on longer exposures of this immunoblot (data not shown). However, by comparison with lane 7, it appears that lower levels of immunoreactive HA-CK2α relative to immunoreactive HA-CK2β are evident in lane 8. Furthermore, in similar experiments, HA-CK2α was not consistently detected in complexes containing Myc-CK2β1–166 and HA-CK2β. We do not have a precise explanation for the apparent low levels of HA-CK2α that are observed in these complexes. However, the apparent low levels of HA-CK2α that are observed in complexes with Myc-CK2β1–166 in lane 8 may in part result from the expected lower stoichiometry of HA-CK2α in complexes with Myc-CK2β1–166 (i.e. expected to be one catalytic subunit per complex) as compared with complexes with full-length Myc-CK2β.
CKβ (i.e. expected to be two catalytic subunits per complex). Alternatively, it is possible that two intact CKβ subunits are necessary for the formation of complexes with catalytic CK2 subunits that are stable under the conditions of immunoprecipitation used in this study.

In the experiments shown in Fig. 6, the detection of Myc-CKβ1–166 in anti-Myc immunoprecipitates was confounded by the light chain of IgG (data not shown). Therefore, as an alternative to immunoblots, we also performed biosynthetic labeling experiments. As shown in Fig. 7, with full-length Myc-CKβ (Fig. 7A), autophosphorylation is evident when it is co-expressed with either HA-CK2α (lane 2) or with a combination of HA-CK2α and HA-CK2β (lane 4). Autophosphorylation of Myc-CKβ is not evident when Myc-CKβ is cotransfected with the control vector (lane 1) or with HA-CK2β (lane 3), indicating that the catalytic subunits of CK2 are required for autophosphorylation and that it does not serve as a substrate for endogenous CK2. With Myc-CKβ1–166 (Fig. 7B), autophosphorylation is not evident when it is cotransfected by itself or with either HA-CK2β or HA-CK2α (lanes 1–3). However, autophosphorylation of Myc-CKβ1–166 is evident when co-expressed with HA-CK2β and HA-CK2α′, demonstrating that HA-CK2β promotes autophosphorylation of Myc-CKβ1–166. The shift in electrophoretic mobility that was observed with Myc-CKβ1–166 when co-expressed with HA-CK2α′ and HA-CK2β was not observed when a kinase-inactive mutant of HA-CK2α′ was utilized (data not shown). This result confirms that the shift in mobility of Myc-CKβ1–166 is the result of phosphorylation.

**DISCUSSION**

Through the examination of complexes that are formed between transfected CK2 subunits in Cos-7 cells, we have obtained evidence that complexes between two CKβ subunits can form in cells in the absence of CK2α. These results are consistent with the interpretation that complexes between CK2β subunits precede incorporation of catalytic CK2 subunits into tetrameric complexes. Our previous biosynthetic labeling studies are also consistent with this interpretation (24) because in that study we demonstrated that CKβ2 is synthesized in excess of CK2α. Furthermore, by following the fate of newly synthesized CK2 subunits (24), we demonstrated that CK2α is rapidly incorporated into complexes with CKβ2. By comparison, incorporation of CKβ into complexes with CK2α occurs more slowly. Under some circumstances (i.e. if CK2α were more abundant than CK2β) it may be possible that the assembly of tetrameric CK2 complexes forms by a different mechanism (i.e. CK2α:CK2β heterodimers precede tetramer assembly). However, because our biosynthetic labeling studies suggest that CKβ is synthesized in excess of CK2α (24) and because this study demonstrates that complexes between CKβ subunits form in the absence of catalytic subunits, we believe that the most likely interpretation of the data is that formation of CKβ complexes precedes incorporation of catalytic CK2 subunits into tetrameric complexes. Importantly, these results suggest that the recent crystal structure of a dimeric form of the regulatory CKβ subunit may indeed represent a physiologically relevant structure (41) because it does not appear from the data presented here that catalytic CK2 subunits are required for the formation of complexes between two CKβ subunits.

By examining the formation of complexes between deletion products of CKβ and full-length CK2α′ or CK2β in cells, we have identified a deletion product of CKβ (i.e. CKβ1–166) that retains the ability to interact with CKβ but fails to stably interact directly with CK2α′. The former results are consistent with the recent crystal structure of a dimeric form of CK2β (41). From that study, it has been demonstrated that interactions between CKβ subunits are mediated by a zinc finger that is composed of Cys309, Cys314, Cys317, and Cys340. Moreover, our results may also have broader implications regarding the assembly of CK2 in cells. Although definitive conclusions may not be possible until detailed three-dimensional structural information for tetrameric CK2 is available, our results indicate that a single intact CKβ subunit is sufficient to recruit a catalytic subunit into a multi-subunit CK2 complex that allows intramolecular autophosphorylation of CKβ.

It is intriguing that full-length HA-CK2β promotes phosphorylation of Myc-CKβ1–166 (Fig. 7). One possible explanation for this observation is that the intramolecular autophosphorylation of CKβ that occurs involves phosphorylation by the catalytic subunit that is associated with the other CKβ subunit (i.e. trans-phosphorylation). This suggestion is based on the observation that Myc-CKβ1–166 does not directly interact with HA-CK2α′. When Myc-CKβ1–166 forms a complex with full-length CK2β, a catalytic CK2 subunit may then be recruited to the complex with a resultant autophosphorylation of Myc-CKβ1–166. However, we have not excluded other possibilities. For example, we do not know the nature of the complexes that form between Myc-CKβ1–166: HA-CK2β and HA-CK2α′. One might expect that these complexes would be trimeric (i.e. containing only one catalytic subunit) because Myc-CKβ1–166 does not directly interact with HA-CK2α′. However, because we observe that full-length HA-CK2β is also phosphorylated in complexes containing Myc-CKβ1–166, it is possible that the one catalytic subunit has sufficient mobility to autophosphorylate both Myc-CKβ1–166 and HA-CK2β or alternatively, it is possible that the complexes do in fact contain two catalytic subunits. Our attempts to resolve this issue by gel filtration on an fast protein liquid chromatography were inconclusive because we observed that complexes that formed between catalytic and regulatory subunits of CK2 migrated as complexes with apparent molecular weights greatly exceeding the expected for trimeric or tetrameric CK2 complexes (data not shown). As recently noted by Guerra and Issinger (42), this observation likely reflects the fact that CK2 interacts with a variety of cellular proteins to form high molecular weight complexes.

Overall, although there are unresolved issues regarding the nature of the complexes that are formed with Myc-CKβ1–166, our data suggest that a single full-length CKβ subunit is sufficient to recruit catalytic subunits into complexes that allow autophosphorylation. It is also interesting to note that a transphosphorylation reaction is responsible for the autophos-
phorylation reactions that are observed upon dimerization of a number of receptor tyrosine kinases (including the insulin, epidermal growth factor, and nerve growth factor receptors) (43–46). Consequently, although we have not excluded other possibilities, it is plausible that the autophosphorylation of a protein serine/threonine kinase such as CK2 is mechanistically similar to that of receptor tyrosine kinases.

As yet, the functional significance of the autophosphorylation of CK2β remains undefined. However, on the basis that kinase-inactive CK2 is expressed in cells at lower levels than enzymatically active CK2, we speculated that autophosphorylation may enhance the stability of CK2 (18). In this regard, it is interesting that we observe with Myc-CK2β enzymatically active CK2, we speculated that autophosphorylation may enhance the stability of CK2 (18). In this regard, it is interesting that we observe with Myc-CK2β or Myc-CK2β1–166 that apparent levels of expression are higher under situations where they are autophosphorylated than under situations where they are not phosphorylated (Fig. 7A, compare lanes 2 and 4 with lanes 1 and 3; Fig. 7B, compare lane 4 with lanes 1–3). However, because autophosphorylation is an apparent marker of complex assembly, our results may simply indicate that complexed subunits are more stable than noncomplexed subunits.

Most of the information regarding the domains of interaction on the subunits of CK2 has been obtained from studies with the yeast two-hybrid system (22, 23, 39). Given the propensity of the two-hybrid system to yield false positives or negatives (47, 48), it is therefore important that results obtained in yeast be confirmed by another means. In this regard, our studies demonstrate that the N-terminal domain of CK2β (i.e. CK2β1–166) interacts with another CK2β but fails to interact directly with a catalytic CK2α subunit. Despite the failure of CK2β1–166 to interact directly with CK2α, there are further indications from a number of studies that this region does play a role in the regulation of CK2 and that it may be in close proximity to CK2α within the CK2 tetramer. In particular, Krehan et al. (37, 38) demonstrated that residues 55–70 of CK2β could be cross-linked to CK2α. Furthermore, acidic residues within the same region have been implicated as the binding sites for polyamines that stimulate CK2 activity (19). These residues may function as an autoinhibitory domain that interacts with the catalytic site of CK2α. In a similar vein, the biochemical experiments of Marin et al. (15) demonstrated that CK2β1–77 potently inhibits the activity of CK2α toward substrates such as calmodulin. However, this fragment of CK2β did not form a stable complex with CK2α. Collectively, these studies suggest that the N-terminal region of CK2β does communicate with CK2α, but this region by itself is not sufficient for stable interactions with CK2α. If the intramolecular autophosphorylation of CK2β did indeed occur through transphosphorylation, the N-terminal region of CK2β may actually interact with the catalytic subunit of CK2 that is stably attached to the other CK2α subunit within the tetramer. Consequently, by mediating the formation of tetrameric CK2 complexes, CK2β can exert the dual regulatory functions that have been described by Marin et al. (15). Although the C-terminal domain of CK2β stably interacts with one catalytic subunit and influences CK2 by stabilizing and stimulating the kinase activity of that catalytic subunit, the N-terminal domain (i.e. negative regulatory domain) of the same CK2β molecule has the potential to interact with and regulate the other catalytic subunit. Stated in another way, a single catalytic subunit may have interactions with both CK2α subunits within the CK2 tetramer; stable interactions require the C-terminal domain of one CK2β, whereas transient interactions involve the N-terminal domain of the other CK2β subunit. Tetrameric complexes of CK2 may therefore be subject to more precise regulation than would be afforded in a complex composed of one regulatory subunit and one catalytic subunit.

Further work will be required to obtain experimental support for this model.

Our results also suggest that the C-terminal region of CK2β is necessary for the direct formation of stable interactions with CK2α because evidence of HA-CK2α was not detected in Myc-CK2β1–166 immunoprecipitates unless full-length CK2β was present. From two-hybrid studies (23), the smallest fragment of CK2β that exhibits the capacity for stable interactions with CK2β encodes residues 152–200. The precise boundaries of the CK2α interaction domain of CK2β and whether a single domain of interaction exists remains to be determined. It is also noteworthy that CK2β has been shown to interact with other protein serine/threonine kinases including A-Raf and c-Mos (49–52). Interestingly, interactions between these enzymes and CK2β also involve the C-terminal domain of CK2β. These results suggest that CK2α, A-Raf, and c-Mos may have common three-dimensional elements that are involved in interactions with a common domain of CK2β.

A detailed understanding of the assembly of CK2 subunits into tetrameric complexes may yield insights for the control of CK2 or its functions in cells. In particular, through the overexpression of a fragment of CK2β such as CK2β1–166, which retains the ability to interact with CK2β but fails to interact directly with the catalytic subunits of CK2, it may be possible to alter the composition of CK2 complexes within cells. These strategies may be valuable for probing possible functions for CK2α and/or CK2β that exist outside of tetrameric CK2 complexes. For example, by preventing the formation of tetrameric complexes between CK2α and CK2β, it may be possible to enhance the activity of CK2β to perform its other functions. Additionally, because the N-terminal region of CK2β has been shown to be important for interactions with CK2 substrates such as Nopp140 (53) and p53 (54), expression of a deletion of CK2β such as CK2β1–166 may offer a strategy for altering the phosphorylation of some cellular CK2 targets.

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The Regulatory β Subunit of Protein Kinase CK2 Mediates Formation of Tetrameric CK2 Complexes

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