*Molecular and Cellular Biochemistry* **191:** 75–83, 1999.


# Interactions of protein kinase CK2 subunits

Iris Korn,[1] Silvio Gutkind,[2] N. Srinivasan,[3] Tom L. Blundell,[3] Catherine C. Allende[1] and Jorge E. Allende[1]

[1]*Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile;* [2]*Laboratory of Cellular Development and Oncology, National Institutes of Dental Research, National Institute of Health, Bethesda, MD, USA;* [3]*Department of Biochemistry, University of Cambridge, Cambridge, UK*

## Abstract

Several approaches have been used to study the interactions of the subunits of protein kinase CK2. The inactive mutant of CK2α that has Asp 156 mutated to Ala (CK2αA$^{156}$) is able to bind the CK2β subunit and to compete effectively in this binding with wild-type subunits α and α′. The interaction between CK2αA$^{156}$ and CK2β was also demonstrated by transfection of epitope-tagged cDNA constructs into COS-7 cells. Immunoprecipitation of epitope-tagged CK2αA$^{156}$ coprecipitated the β subunit and vice-versa. The assay of the CK2 activity of the extracts obtained from cells transiently transfected with these different subunits yielded some surprising results: The CK2 specific phosphorylating activity of these cells transfected with the inactive CK2αA$^{156}$ was considerably higher than the control cells transfected with vectors alone. Assays of the immunoprecipitated CK2αA$^{156}$ expressed in these cells, however, demonstrated that the mutant was indeed inactive. It can be concluded that transfection of the inactive CK2αA$^{156}$ affects the endogenous activity of CK2. Transfection experiments with CK2α and β subunits and CK2αA$^{156}$ were also used to confirm the interaction of CK2 with the general CDK inhibitor p21$^{WAF1/CIP1}$ co-transfected into these cells. Finally a search in the SwissProt databank for proteins with properties similar to those derived from the amino acid composition of CK2β indicated that CK2β is related to protein phosphatase 2A and to other phosphatases as well as to a subunit of some ion-transport ATPases. (Mol Cell Biochem **191**: 75–83, 1999)

*Key words*: protein kinase, CK2, p21, protein phosphatase 2A

## Introduction

Protein kinase CK2 is a multifunctional ser/thr protein kinase composed of two types of subunits: The catalytic α and α′ subunits [Mr 37–44] and the regulatory β subunit [Mr 24–27]. (For reviews see [1–3]) CK2 is composed of $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$ and $\alpha'_2\beta_2$ heterotetramers [4] and also, depending on the ionic conditions, higher order aggregates [5].

The CK2β subunit has several effects on the catalytic activity of α or α′. With most substrates, the β subunit stimulates the phosphorylating activity of α and α′ by 5–20 fold [6]. However, with a few substrates such as calmodulin [7, 8] or MDM-2 [9], the β subunit inhibits the phosphorylating activity of the catalytic subunit. In addition, CK2β protects the catalytic subunits α and α′ from heat and proteolytic degradation and shifts upward the salt concentration required for optimal activity. The β subunit also is responsible for the stimulation of CK2 phosphorylation by polyamines [10] and causes the CK2α subunit to decrease its binding to DNA [11].

A large number of studies have used site-directed mutagenesis and the yeast two-hybrid system to explore the regions of interaction between the CK2α and β subunits. The conclusions of these studies are that the carboxyl region of CK2β (amino acids 171–194) plays a key role in the binding of the α subunit [12]. It was also observed that CK2β dimerizes and the central part of the molecule appears to be involved in that interaction [13]. The results obtained with CK2α mutants and with antibodies are not as clear, but they suggest some involvement of the basic region (amino acids 74–81) in its interaction with CK2β [14].

Recent findings, however, have shown that the β subunit of CK2 also interacts with several other proteins as shown

*Address for offprints*: J.E. Allende, Programa de Biología Celular y Molecular, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Casilla 70086, Santiago 7, Chile

in Fig. 1. CK2β, either free or as part of the holoenzyme, binds to p53 and affects the DNA re-annealing activity of this key tumor-suppressor protein [15]. The β subunit was found also to bind the $p21^{WAF1/CIP1}$ inhibitor of the cyclin dependent protein kinases. The presence of p21 was found to inhibit CK2 phosphorylation of casein and of the β subunit itself [16]. The interaction of $p21^{WAF1/CIP1}$ with CK2β seems also to occur when this subunit is either free or bound to the α subunit. A different kind of interaction has been observed with the free CK2β subunit. It has been demonstrated that A-Raf binds to CK2β and its kinase activity seems to be stimulated by this interaction [17, 18]. The A-Raf binding of β is competitive with CK2α. A similar observation has been reported with c-Mos, an oncogenic protein kinase that, like the Raf family, can phosphorylate MEK and activate the MAP cascade that signals cell division [19]. In this case, however, it is reported that CK2β inhibits the activity of c-Mos.

A recent report demonstrates that CK2α interacts with and phosphorylates the catalytic subunit of protein phosphatase 2A [20]. This interaction is blocked by the presence of CK2β and activates moderately the dephosphorylation of MEK by PP2A.

The CK2 subunits, therefore, are promiscuous in that they can interact with several other proteins that play important roles in the transduction of the cell division signals. In general these interactions of CK2 subunits with other proteins have been demonstrated only in the yeast two-hybrid system or *in vitro* and their physiological role has not been established.

In our laboratory we have undertaken the study of the role of CK2β *in vivo* by the designing of an inactive mutant of CK2α that could trap the β subunit in a dead complex. This type of 'dominant negative' mutant ($CK2\alpha A^{156}$) has been designed and confirmed to interact with CK2β with high affinity *in vitro* [21].

In the present report, we have studied further this $CK2\alpha A^{156}$ mutant and have shown that it can bind CK2β when expressed in transfected COS-7 cells. Paradoxically, however, the CK2 activity of these mutant-transfected cells appears to increase. In addition, the interaction of transfected CK2α, $CK2\alpha A^{156}$ and CK2β with $p21^{WAF1/CIP1}$ is also demonstrated. We also present a computer analysis which indicates that there may be a structural and/or functional relatedness between the β subunit and the catalytic subunit of protein phosphatase 2A, as well as to some subunits of ion transport ATPases.

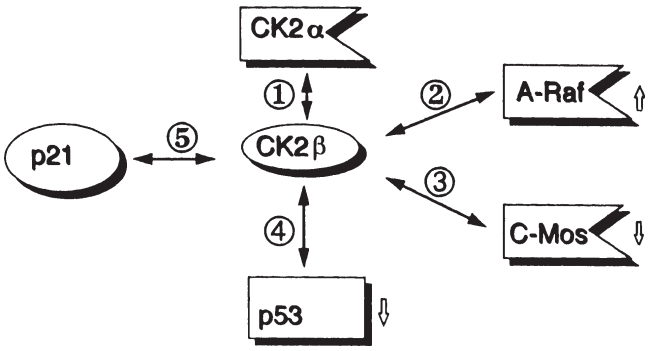


*Fig. 1.* CK2β subunit interacts with several proteins. In reactions 2 and 3, the CK2α subunit interaction with CK2β is competitive with that of the other proteins. For reactions 4 and 5, interaction may occur with both CK2β alone or with $CK2\alpha_2\beta_2$.

## Materials and methods

*Expression plasmids*

The cDNAs coding for the subunits CK2α, α′, and $\alpha'^{156}$ subcloned in NdeI and SalI sites of pT7-7H6 were obtained as described [22]. For subcloning into the eucariotic expression vector pCEFL (a modified pEBG plasmid, [23]) , BglII and NotI sites were generated in the pT7-7H6 vector, replacing the NdeI and SalI sites, and the coding sequences were excised by restriction digestion at these sites. The cDNAs were then inserted into the pCEFL vector containing the epitope tag for influenza hemagglutinin (HA) [24] or for the AU5 hexapeptide [25]. For subcloning of the CK2β subunit, the previously described pGEX-2T expression vector containing CK2β cDNA [26] was digested with EcoRI and subcloned in pCDNA3 (Invitrogen) to change the reading frame, then digested with BamHI and NotI and subsequently cloned in the BglI and NotI sites of modified pCEFL expression plasmid, encoding an epitope tag as given above. p21 protein was expressed from vector pCDNA3.

*Transfection of COS-7 cells*

Subconfluent COS-7 cells were transfected or cotransfected with expression vector alone or carrying cDNAs coding for wail type CK2 subunits, $CK2\alpha A^{156}$ and/or p21 (1 μg DNA per plate in each case) by the DEAE-dextran technique [27]. Cells were grown at 37°C in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells were harvested at 48 h post-transfection. Transcription efficiency was controlled by a parallel transfection of cells with 1 μg of pCMV-βgal, checking the β-galactosidase activity colorimetrically.

Cell lysates were prepared by washing transfected plates with phosphate buffered saline (PBS) and cells scraped into 950 μl lysate buffer containing 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 1 mM PMSF, 2 mM orthovanadate and 20 μg/μl each aprotinin and leupeptin. Lysates were centrifuged at 20,000 × g for 10 min and the supernatant fractions were analyzed the same day or stored without prior centrifugation at –70°C. Protein was measured by the method of Bradford [28].

*Immunoprecipitations and Western blots*

Epitope-tagged proteins from lysates were incubated with the specific antibody overnight at 4°C and immunoprecipitates recovered in the presence of Gamma Bind G Sepharose (Pharmacia). Precipitates were washed three times in PBS containing 1% NP-40, once with 0.1 M Tris-HCl pH 7.5, 0.5 M LiCl and finally, in the case of kinase reactions, with a buffer containing 50 mM Hepes pH 7.5, 10 mM $MgCl_2$ and 5 mM dithiothreitol or in the case of Western analysis, samples were diluted 5 fold in the gel sample buffer.

Western blots were performed on immunoprecipitates obtained with HA12CA5 and AU5 antibodies (Babco). These immunoprecipitates were dissolved in an equal volume of Laemmli denaturing buffer containing 2-mercaptoethanol and heated at 100°C for 5 min. Proteins were resolved by SDS-polyacrylamide gel electrophoresis (PAGE) as described by Laemmli [29]. Proteins were transferred to nitrocellulose at 150 mA for 5 h, and the nitrocellulose blocked for 2 h at room temperature with 4% bovine serum albumin in Tris-HCl pH 7.5,130 mM NaCl and 0.05% Tween 20. Immunoblotting was carried out with the corresponding mouse monoclonal antibody, and visualization by enhanced chemiluminescence detection (Amersham) using goat anti-mouse antiserum coupled to horseradish peroxidase as secondary antibody.

*Protein kinase CK2 assays*

For assay of immunoprecipitates, 1 mg of protein from total cell extracts was immunoprecipitated and then assayed in a final volume of 50 µl containing 50 mM Hepes, pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, 0.5mM dithiothreitol 100 µM [$\gamma^{32}$P] ATP (sp. act. 4000 cpm/pmol) and 5 mg/ml casein. Incubations were for 10 min at 30°C. The reactions were stopped by the addition of gel sample buffer for analysis on 10% SDS-PAGE. For the assay of CK2 activity in total cell extracts, 5 µg of extract protein was used and 1 mM CK2-specific synthetic peptide as substrate, without casein and with the remaining conditions as above. The reaction was stopped by absorbing the reaction mixture onto 1.5 $cm^2$ pieces of P81 phosphocellulose paper and processing as described previously [22]. The assays of the activity of recombinant CK2 subunits *in vitro* was performed as with extracts but using the amounts indicated in each case and a 30 µl volume assay with 100 mM NaCl and casein as substrate. All assays were performed in duplicate and for each assay, a control was performed in the presence of 5 µg/ml heparin and another in the absence of substrate. The kinase activity values presented are corrected for the amount of incorporation observed in the presence of 5 µg/ml heparin, which was always less than 5% of that observed in its absence. The linearity of the reaction was determined in each case.

The source of other products used was as follows: Anti-Hemagglutin (HA) 12CA5 and anti-AU5 (hexapeptide epitope) monoclonal antibodies were from Babco; anti-CDK2 monoclonal antibody from Santa Cruz; Dulbecco's modified Eagle's medium and trypsin from Life Technologies; G-protein Sepharose from Pharmacia; [$\gamma$-$^{32}$P] ATP from New England Nuclear; P8l phosphocellulose from Whatman, and all other reagents from Sigma Chemical. The CK2 specific synthetic peptide (RRRDDDSEDD) was synthesized by Oligopeptido, the University of Chile core facility.

*Sequence analysis of CK2β*

The Propsearch analysis [30] was carried out from the Swiss-Prot databank and the computer print-out was selected for proteins of special interest. This procedure considers amino acid composition at the singlet and doublet levels, molecular weight, polarity of amino acids and isoelectric point of the given sequence and many other properties and compares these with corresponding properties of all the proteins in the sequence database.

*Secondary structure prediction and threading*

Multiple sequence alignment of all known β -subunits has been used in prediction of secondary structure and solvent accessibility classes using *SAPIENS* [31, 32] and *SPECTOR* [33].

# Results

*The* in vitro *competition of a mutant CK2α form with CK2α and α′*

The interaction of the CK2 α and β subunits has been shown to be inhibited *in vitro* by the presence of a catalytically inactive mutant form of CK2α, CK2αA$^{156}$ [21]. However, the tissue specific expression of more than one isoform of the CK2α subunit (α and α′) made it pertinent to determine if this mutant can also compete for CK2β with the α′ isoform. The native CK2 α and α′ isoforms possess significantly different characteristics, specifically the presence of 41 additional amino acids at the carboxyl terminal region of CK2α as well as different isoelectric points and salt sensibility [6]. In Fig. 2 it is shown that the *in vitro* interaction of CK2β with both α and α′ isoforms is inhibited by the presence of CK2αA$^{156}$. In this experiment the amount of regulatory CK2β was limiting with respect to α and α′, however the stimulation of basal activity by CK2β was over eight-fold in both cases. The addition of increasing concentrations of the inactive mutant CK2αA$^{156}$ results in the progres-

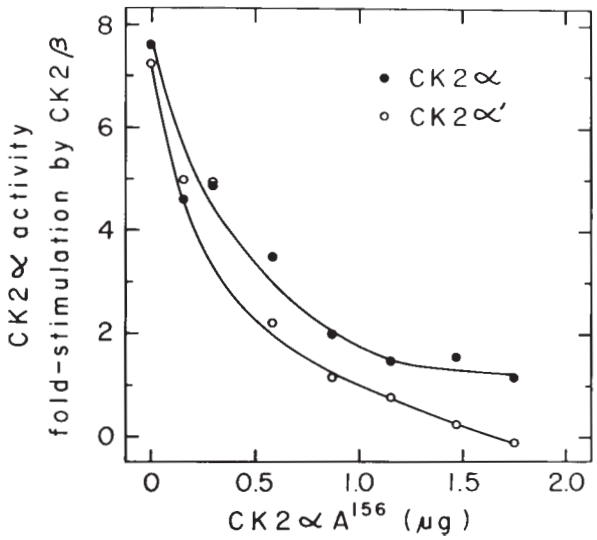


*Fig. 2.* CK2αA$^{156}$ inhibits the activation of the α and α′ subunits of CK2. Varying concentrations of CK2αA$^{156}$ were used in the presence of 1 pmol of either CK2α or α′ and 0.35 pmol of CK2β for assay under standard conditions using casein as substrates. The basal activities of CK2α and α′ were 1.1 and 0.95 pmol/min, respectively and the fold-stimulation by CK2β were 8.6 (α) and 8.2 (α′).

sive loss of the stimulation caused by CK2β, showing that both CK2α isoforms are affected to a similar extent in their interaction with CK2β, although the inhibition of α′ is slightly more pronounced indicating that its affinity for β might be less than that of CK2 α.

### *Interaction of CK2αA$^{156}$ with CK2β in transfected cells*

In order to investigate the interaction of the CK2β with the inactive CK2α mutant in intact cells, hemagglutinin (HA) epitope-tagged CK2α, CK2β and CK2αA$^{156}$ were expressed in COS-7 cells. As shown in Fig. 3A, all three proteins were expressed at easily detectable levels as determined by Western blotting of total cell lysates using an anti-HA monoclonal antibody.

Cotransfection experiments which utilized both a HA-tagged vector as well as an alternative epitope-tagged expression vector (AU5) permitted the immunodetection of CK2 subunits which interact in these cells. Cells cotransfected with AU5-CK2α and HA-CK2β were immunoprecipitated with anti AU5 monoclonal antibody and the proteins resolved by gel electrophoresis. Western blotting with anti-HA re-

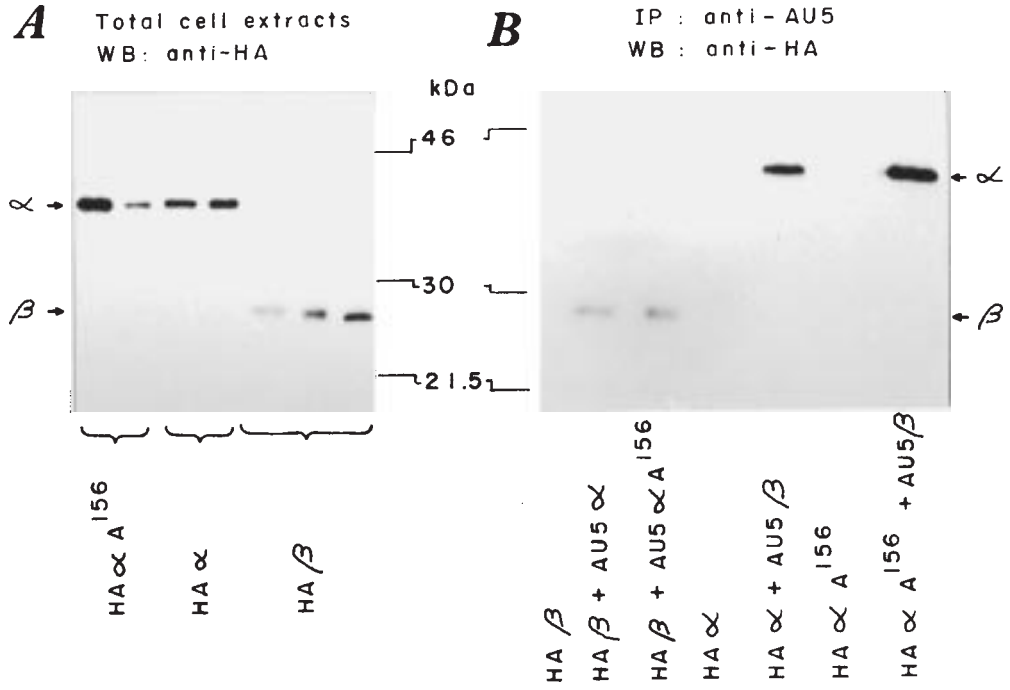


*Fig. 3.* Western analysis of cell extracts from COS-7 cells overexpressing CK2 subunits. (A) COS-7 cells were transfected with pCEFL-HAα, pCEFL-HAαA$^{156}$ or pCEFL-HAβ constructs and extracts prepared as given in Materials and methods. Proteins were resolved on 12% polyacrylamide gels. Western analysis using anti-HA antibody and gel electrophoresis were performed as given in Materials and methods. The different lanes show results using extracts from separate transfection experiments. (B) Cells were transfected with pCEFL-AU5α, pCEFL-AU5αA$^{156}$ or pCEFL-AU5β and with the HA constructs as indicated. Cell extracts were immunoprecipitated using anti-AU5 monoclonal antibody and precipitates processed by gel electrophoresis and Western blot. Specific bands detected by the anti-HA and AU5 antibodies as well as molecular mass standards are indicated.

vealed the presence of the CKβ (Fig. 3B, second lane), indicating the coprecipitation of these subunits, as expected. When the cotransfection was carried out with the AU5-CK2αA$^{156}$ and HA-CK2β (third lane) the presence of the β subunit shows that the mutant subunit is equally capable of coprecipitating this regulatory protein.

The expression of AU5-tagged-CK2β (lanes 5 and 7) and cotransfection with the CK2α subunits as HA-tagged proteins showed that both native and mutant CK2α subunits co-immunoprecipitated the expressed β subunit. These experiments indicate that the interaction of the mutant subunit is as efficient as the wild type CK2α subunit in recognizing the endogenously expressed CK2β subunit.

*The effect of CK2αA$^{156}$ on CK2 activity in transfected cells*

The data presented in Fig. 4 represents studies of the functionality of the expressed CK2 subunits in the supernatant fraction of total lysates of cells that have been transfected as described in the previous section. The experiment is representative of five separate analyses of transfected cells.

Part A of Fig. 4 presents the results obtained by assaying directly the CK2 activity of the lysate supernatant fractions using the specific CK2 peptide substrate RRRDDDSEDD. The endogenous CK2 activity obtained with cells transfected with vector without a CK2 insert is low. As expected, transfection with CK2α alone or with both CK2α and CK2β causes a large increase in the activity of the lysates. CK2β transfection also causes an important increase. The surprising result observed, inactive CK2αA$^{156}$ however, is that transfection with the inactive CK2αA$^{156}$ either alone or in combination with CK2β also causes increases in CK2 activity that are similar to those observed with CK2α or CK2β alone. In Fig. 4B, CK2 activity is measured in immunoprecipitates using anti-HA, obtained from the same lysates assayed above (Fig. 4A) except that the immunoprecipitated proteins were incubated with [γ$^{32}$P]ATP and casein as substrate. The phosphorylated casein was detected by autoradiographs of the products separated on SDS-PAGE. In this case, the results contrast with those obtained with whole extracts in that significant activity was detected in immunoprecipitates from cells transfected with CK2α and with both α and β subunits but not with the inactive CK2αA$^{156}$ mutant. The immunoprecipitate of the cells transfected with only HA-β subunit had more activity than those of those transfected with the CK2αA$^{156}$.

*The interaction of CK2 subunits with p21$^{WAF1/CIP1}$*

The transfections of cells with the various CK2 subunits and the inactive CK2αA$^{156}$ provided the opportunity to test their interaction with another important regulatory protein under

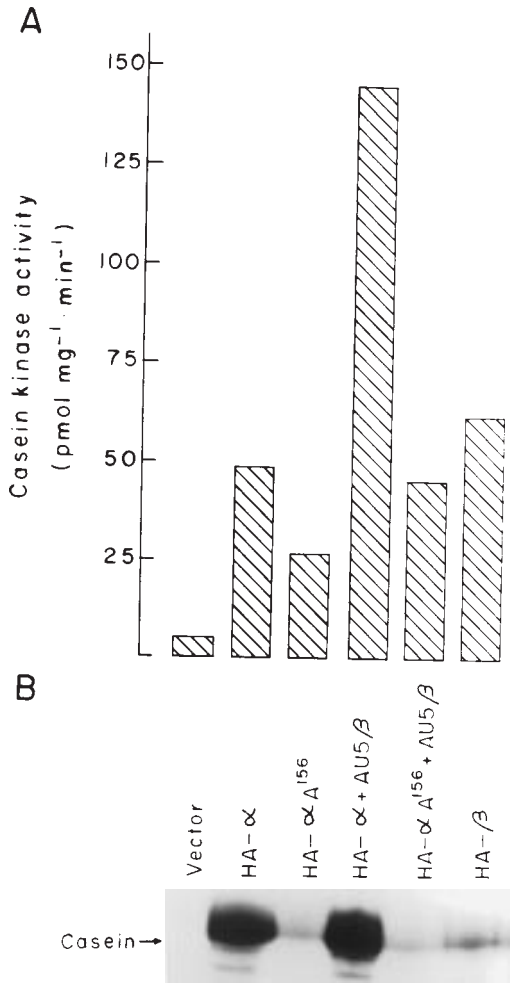


*Fig. 4.* CK2 activity in cell extracts and immunoprecipitates of transfected cells. Cos-7 cells were transfected with the indicated expression plasmid for epitope-tagged CK2α, CK2αA$^{156}$ and/or CK2β, or with plasmid without insert (lane 1) and assayed. (A) Cell extracts were prepared and assays performed as given in Materials and methods, using the specific peptide substrate RRRDDDSEDD. Activity levels are corrected for incorporation in the presence of 5 μg/ml heparin. (B) Immunoprecipitates were obtained using the monoclonal antibody HA12CA5 and 1 mg protein from the same cell extracts as used in A. and assayed using casein as substrate as given in Materials and methods. The experiment is representative of five similar analyses.

conditions that approach the *in vivo* situation. The interaction of p21$^{WAF1/CIP1}$ with CK2β had been demonstrated under strictly *in vitro* conditions with purified proteins [16].

The results shown in Fig. 5 demonstrate that immunoprecipitation of CK2β (lane 2), CK2α (lane 3), CK2αA$^{156}$ (lane 4) as well as of endogenous CDK2 (lane 5) can bring down HA-p21$^{WAF1/CIP1}$ that can be detected by Western blot. Densitometry of the p21$^{WAF1/CIP1}$ bands demonstrates that the amount of the protein immunoprecipitated with the transfected wild-type α and β subunits is considerably lower than that observed with αA$^{156}$ mutant and with CDK2. This latter

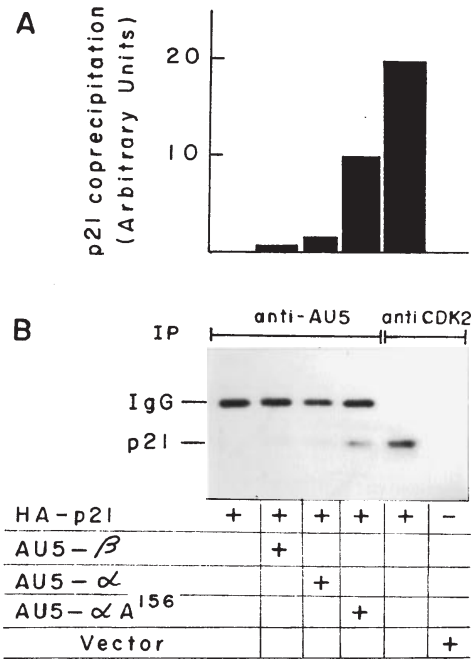


*Fig. 5*. Effect of coexpression of CK2 subunits and epitope-tagged HA-p21. The coexpression of AU5-CK2α, AU5-CK2β, AU5-CK2αA$^{156}$ with HA-p21 in the respective expression plasmids or vector without insert was carried out in COS-7 cells and cell lysates immunoprecipitaded with anti-AU5 or anti CDK2 monoclonal antibody as described in Materials and methods. Proteins were resolved on SDS-polyacrylamide gels and subjected to immunoblotting using anti-HA antibody. (A) Arbitrary units obtained by densitometry of bands detected for migration of p21. (B) Western blot of immunoprecipitated cell lysates using anti-HA (arrow). The slower migrating bands correspond to non-specific immunoglobulin.

immunoprecipitation was performed as a positive control since CDK2/p21$^{WAF1/CIP1}$ interaction is well established.

### *Relatedness of CK2β to protein phosphatase 2A*

Computer analysis of the sequence of CK2β does not show any significant similarity of this protein to other proteins in the databank with the exception of the *stellate* protein from *Drosophila* which has been shown to replace β in the activation of CK2α [34].

However, a program that is not based in direct sequence comparison but rather on the aggregate analysis of many properties and characteristics of the proteins such as size, amino acid composition, isoelectric points and frequency of amino acid pairs yields rather interesting results. The Propsearch program [30] revealed a high similarity between the CK2β subunits and some protein phosphatases and ion transport ATPases. The size (in terms of number of amino acids and the molecular mass) of CK2β variants is very similar for homologous CK2β and for the catalytic subunit of PP2A. The β subunit of the $K^+/H^+$ ATPase is also of similar size. As seen in Table 1, in a search for *Xenopus* CK2β, the top 8 rankings of similar proteins comprised isoforms of β subunit and these corresponded to Propsearch distances in the range 1.85–10.63 which provide a confidence level of 80–

*Table 1.* Occurrence of isoforms of CK2β and various phosphatases and ion transport ATPases during a search for *Xenopus* CK2β using PROPSEARCH

| Rank | Protein identification code | CK2β,phosphatase or Ion ATPase | PROPSEARCH distance | Confidence factor (in %) |
|---|---|---|---|---|
| 1 | kc2b_xenia | CK2β | 1.85 | 99.6 |
| 2 | kc2b-human | CK2β | 1.88 | 99.6 |
| 3 | kc2b-bovin | CK2β | 2.79 | 99.6 |
| 4 | kc2b_drome | CK2β | 3.64 | 99.6 |
| 5 | kc2b_schpo | CK2β | 8.73 | 87.0 |
| 6 | kc2b_caeel | CK2β | 10.06 | 80.0 |
| 7 | kc2b_arath | CK2β | 10.63 | 80.0 |
| 8 | S47968 | CK2β | 10.63 | 80.0 |
| 10 | ptp_npvac | Phosphatase | 10.97 | 80.0 |
| 11 | JH0480 | Ion ATPase | 11.06 | 80.0 |
| 12 | p2a3_qrqth | Phosphatase | 11.17 | 80.0 |
| 17 | A54907 | CK2β | 11.57 | 68.0 |
| 18 | kc2c_yeast | CK2β | 11.57 | 68.0 |
| 19 | S52659 | Phosphatase | 11.59 | 68.0 |
| 21 | p2a_brana | Phosphatase | 11.64 | 68.0 |
| 22 | athb_pig | Ion ATPase | 11.75 | 68.0 |
| 23 | p2a_medsa | Phosphatase | 11.84 | 68.0 |
| 28 | p2a_pig | Phosphatase | 11.95 | 68.0 |
| 29 | p2al_schpo | Phosphatase | 11.96 | 68.0 |
| 30 | p2aa_rat | Phosphatase | 11.99 | 68.0 |
| 32 | p2aa_human | Phosphatase | 12.01 | 68.0 |
| 35 | p2ab_human | Phosphatase | 12.11 | 68.0 |
| 39 | p2a2_arath | Phosphatase | 12.15 | 68.0 |
| 40 | p2a1_arath | Phosphatase | 12.17 | 68.0 |
| 41 | p2qb_rabit | Phosphatase | 12.17 | 68.0 |
| 45 | S09378 | Phosphatase | 12.25 | 68.0 |
| 48 | S20348 | Phosphatase | 12.30 | 68.0 |
| 49 | athb_rabit | Ion ATPase | 12.31 | 68.0 |
| 59 | pp11_yeast | Phosphatase | 12.48 | 68.0 |
| 64 | p2a4_yeast | Phosphatase | 12.55 | 53.0 |
| 65 | S37086 | Phosphatase | 12.56 | 53.0 |
| 66 | athb_rat | Ion ATPase | 12.56 | 53.0 |
| 70 | B55346 | Phosphatase | 12.62 | 53.0 |
| 71 | A40993 | Ion ATPase | 12.65 | 53.0 |
| 77 | pp11_schpo | Phosphatase | 12.74 | 53.0 |
| 92 | p2a_drome | Phosphatase | 12.83 | 53.0 |
| 93 | ppel_schpo | Phosphatase | 12.84 | 53.0 |
| 95 | kc2b_yeast | CK2β | 12.85 | 53.0 |
| 119 | S47967 | CK2β | 13.04 | 53.0 |
| 120 | kc2b_arath | CK2β | 13.04 | 53.0 |
| 123 | S11062 | Phosphatase | 13.05 | 53.0 |
| 127 | atnd_bufma | Ion ATPase | 13.07 | 53.0 |
| 144 | ppx_rabit | Phosphatase | 13.14 | 53.0 |
| 150 | ppx_human | Phosphatase | 13.17 | 53.0 |
| 156 | p2a1_yeast | Phosphatase | 13.18 | 53.0 |
| 158 | S42558 | Phosphatase | 13.19 | 53.0 |
| 171 | kka1_yeast | Phosphatase | 13.27 | 53.0 |

99.6% of relatedness (Table 1). Interestingly the shortest Propsearch distance of a phosphatase with β subunits is 10.97 corresponding to the 80% confidence level. Five homologous β-subunits have considerably lower ranks compared to many phosphatases or ion channel ATPase subunits. Out of the first 50 positions in the ranking, 15 positions are occupied by phosphatases, especially of the PP2A type. Results of Propsearch for other isoforms of CK2β are similar to the one for *X. laevis* CK2β. Results of secondary structure prediction using different programs are consistent and a typical result is shown in Fig. 6. CK2β subunits are predicted to contain both α-helices and β-strands which are roughly alternating. This mixed α and β secondary structure predicted for CK2β is consistent with the fold of phosphatases [35] where a central β-sheet is surrounded by α-helices.

## Discussion

As a result of the recent advances in modern biology the idea of 'one protein - one function', which for many years held sway in our thinking, has turned untenable. It has become more the norm than the exception that proteins fulfill many functions and that each protein interacts with and modifies the activity of many other proteins and other biomolecules. This appears to be true for protein kinase CK2 and its subunits. This promiscuity and polyfunctionality of CK2 make the enzyme more interesting, but also it makes it more difficult to define the roles that the CK2 protein and its subunits may be fulfilling *in vivo* under specific sets of circumstances.

Dominant-negative mutants that compete with the wild type proteins for limiting factors or co-factors have been very useful in furthering our understanding of several proteins that act in signal transduction pathways [36]. For this reason, it seemed interesting to attempt to generate a dominant-negative type of mutation for CK2. Some progress has been achieved with the mutant CK2αA$^{156}$ . This mutation that changes the catalytic aspartic acid in position 156 for alanine is catalytically inactive but retains its capacity to bind the CK2β subunit with an affinity similar to that of the wild-type alpha subunit [21]. This mutation has also served to show that, once formed, the $\alpha_2\beta_2$ tetramers do not dissociate to a detectable extent.

The results obtained previously had shown that the αA$^{156}$ mutant could compete with the wild-type α. In the present report, we demonstrate that it also can compete with the α′ subunit. In this respect the αA$^{156}$ has served to compare the relative affinities of α and α′ for β. This type of experiment can be used to analyze the affinities of other α mutants towards the CK2β subunit.

Transfection with tagged mutant and wild-type subunits followed by immunoprecipitation of the extracts of the transfected cells demonstrates interaction of the αA$^{156}$ subunit with the transfected β subunit. This is strong evidence that indicates that the mutant α subunit can also trap free CK2β in inactive complexes under *in vivo* conditions. The results obtained when the CK2 activity is measured in the transfected cells are rather intriguing. Immunoprecipitates of the transfected subunits phosphorylate casein at levels very close to what would be expected. The wild-type α subunit is very active and the activity is enhanced when co-transfected with

```
                        10        20        30        40        50        60
                        |         |         |         |         |         |
Amino acid seq.  MSSSEEVSWISWFCGLRGNEFFCEVDEDYIQDKFNLTGLNEQVPHYRQALDMILDLEPDE
Sec. Str. Pred.  EEEEE         EEE     HHH  E           HHHHHHHHHHH
Acc. pred.       eeeeee...b.bb.......bbb.b.ee.b...b.....eee.ee..ebb.bbbe.eeee

                        70        80        90        100       110       120
                        |         |         |         |         |         |
Amino acid seq.  ELEDNPNQSDLIEQAAEMLYGLIHARYILTNRGIAQMLEKYQQGDFGYCPRVYCENQPML
Sec. Str. Pred.          HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH        EEE
Acc. pred.       eeeeeeeeee.beebbe.b.bbbbb.bbb..ebb..b.ee.ee.....bb.b.bee.e..

                        130       140       150       160       170       180
                        |         |         |         |         |         |
Amino acid seq.  PIGLSDIPGEAMVKLYCPKCMDVYTPKSSRHHHTDGAYFGTGFPHMLFMVHPEYRPKRPA
Sec. Str. Pred.   E         EEEEEE                                HHHHHEE
Acc. pred.       ...b...e.e..b.b.b.eb.e....e.ee.....bb.bb.....bbb....e..eeeee

                        190       200       210
                        |         |         |
Amino acid seq.  NQFVPRLYGFKIHPMAYQLQLQAASNFKSPVKTMR
Sec. Str. Pred.  EEEEE   HHHHHHHHHHHHHHHHH
Acc. pred.       ee....b.bb.b.e.b.e.e....ee.eeeeee.e
```

*Fig. 6.* A representative secondary structure prediction for PKCK2β. This prediction is for the variant from *Xenopus*, but, by using multiple sequence alignment in the PHD procedure [32].

the β subunit. The immunoprecipitates of CK2αA$^{156}$ are practically inactive. The slight activity of these latter immunoprecipitates might be due to formation of mixed tetramers containing endogenous wild-type and αA$^{156}$ alpha subunits. The higher activity of the immunoprecipitates transfected with β would indicate that this transfected subunit is binding to some endogenous α subunit.

The interpretation of the results of the direct assay of CK2 activity in the extracts of the transfected cells is more complex. The CK2 activity detected in cells transfected with the wild-type α and β subunits fits the expected results since it is considerably higher than the cells assayed in the extracts of cells transfected with the control vector without CK2 inserts. (This control CK2 activity coincides with the values obtained with COS-7 cells that have not been transfected (not shown)).

The fact that transfection with β subunit results in higher CK2 activity confirms a previous observation [37] and indicates that CK2β might be limiting in the nontransfected cells. This is relevant because it has been shown that CK2β is synthesized in a large excess over CK2α and would suggest that β might be physiologically bound to other proteins in addition to undergoing rapid degradation [38]

The observation obtained with the cells transfected with the inactive mutant CK2αA$^{156}$ was unpredictable. Contrary to the expected effect of a dominant-negative mutant, the CK2 activity of these cells was found to be considerably higher than that of control cells transfected with the empty vector. Since the results on the activity of the immunoprecipitates indicate that the mutant protein does not become activated when expressed in the cells, the only conclusion that can be derived from the observed increased activity is that the transfection of CK2αA$^{156}$ causes an increase of the activity of the endogenous CK2 in these cells. This increase of activity might be due to the removal of an inhibitor by the presence of inactive CK2αA$^{156}$. In this respect it is interesting to note that inactive CK2α has been shown to bind PP2A [20]. The other possibility is that the presence of CK2αA$^{156}$ might cause the induction of the expression of CK2 subunits. Since these cells are assayed 48 h after the transfection, such an induction could occur during this period. The transfection of CK2α subunits has been found to induce the transcription of CK2β (W. Pyerin, personal communication). Obviously, these unexpected results require further analysis and experimentation.

The work presented here confirm the observation of Götz *et al*. [16] who showed interaction of p21$^{WAF1/CIP1}$ with CK2. These observations, however, extend the previous results since they demonstrate that the interaction can occur under conditions closer to the *in vivo* environment and that p21$^{WAF1/CIP1}$ can be brought down by antibodies against either the α or β subunits and also that p21$^{WAF1/CIP1}$ can bind to inactive CK2 complexes formed with CK2αA$^{156}$. These findings are significant because p21$^{WAF1/CIP1}$ has been shown to inhibit CK2 activity and, therefore, it is possible that this general cdk inhibitor which is induced by p53 might also have a role in the regulation of CK2 function.

The results of the Propsearch, a computer program designed to detect related proteins, are noteworthy because they provide a structural support for an observation which has recently appeared in the literature, the binding of CK2α to protein phosphatase 2A. Recently Hériché *et al*. [20] have demonstrated that CK2α is regulated by phosphatase 2A. Remarkably, phosphatase 2A is shown not only to interact directly with CK2α but it is phosphorylated by CK2α. Most significantly, phosphatase 2A associates with CK2α only in the free form but not when CK2α exists as a holoenzyme in association with CK2β. This suggests that CK2β and phosphatase 2A competitively bind to CK2α implying the possibility of a grossly similar tertiary fold or a similar local structure at the binding regions. Phosphatase 2A is phosphorylated in a dimeric form. This is similar to the situation suggested for the quaternary structure of PKCK2 holoenzyme which is comprised of two copies of CK2β [5]. The CK2β binding site in CK2α is suggested to include the residues 166–171 (*Xenopus* CK2α numbering). This region has been mapped in a modelled tertiary structure of CK2α (Srinivasan *et al*., submitted for publication) where it is located in the region linking two lobes but on the side opposite to the activation loop of the kinase domain. Thus it does not hinder the access of substrates to the kinase and is proximal to the ATP binding region. It is of interest that in the recently described crystal structure of *src* and *hck* kinases [39, 40] the regulator or adaptor domain binding region (SH3 in this case) coincides spacially with the site suggested for of CK2α to β subunit.

The same Propsearch program shows CK2β to be related to subunits of ion transport ATPases found in animal cells. This relatedness is tantalizing because the CK2β subunit has been shown by Glover's group to be involved in ion transport in yeast [41].

## Acknowledgements

This work was supported by grants from the Council for Tobacco Research, the International Centre for Genetic Engineering and Biotechnology and FONDECYT-Chile to Jorge E. Allende and from the Welcome Trust to Tom L. Blundell. Iris Korn, a doctoral student of the Universität Konstanz, is supported by a doctoral grant from the Deutscher Akademischer Auslandsdienst.

## References

1. Pinna LA: Casein kinase 2: An 'eminence grise' in cellular regulation. Minireview-Biochem Biophys Acta 1054: 267–284, 1990

2. Issinger O-G: Casein kinases: Pleiotropic mediators of cellular regulation. Pharmac Ther 59: 1–30, 1993
3. Allende JE, Allende CC: Protein kinase CK2: An enzyme with multiple substrates and a puzzling regulation - Invited review. FASEB J 9: 313–323, 1995
4. Chester N, Yu IJ, Marshak DR: Identification and characterization of protein kinase CKII isoforms in HeLa cells. Isoform-specific differences in rates of assembly from catalytic and regulatory subunits. J Biol Chem 270: 7501–7514, 1995
5. Valero E, De Bonis S, Filhol O, Wade RH, Langowski J, Chambaz EM, Cochet C: Quaternary Structure of Casein Kinase 2. Characterization of multiple oligomeric states and relation with its catalytic activity. J Biol Chem 270: 8345–8352, 1995
6. Antonelli M, Daniotti JL, Rojo D, Allende CC, Allende JE: Cloning, expression and properties of the α′subunit of casein kinase 2 from Zebrafish (*Danio rerio*). Eur J Biochem 241: 272–279, 1996
7. Meggio F, Boldyreff B, Issinger O-G, Pinna LA: Casein Kinase 2 down-regulation and activation by polybasic peptides are mediated by acidic residues in the 55–64 region of the beta-subunit. A study with calmodulin as phosphorylatable substrate. Biochemistry 33: 4336–4342, 1994
8. Bidwai AP, Reed JC, Glover CVC: Phosphorylation of calmodulin by the catalytic subunit of casein kinase II is inhibited by the regulatory subunit. Arch Biochem Biophys 300: 265–270, 1993
9. Guerra B, Götz C, Wagner P, Montenarh M, Issinger O-G: The carboxy terminus of p53 mimics the polylysine effect of protein kinase CK2-catalyzed MDM2 phosphorylation. Oncogene 15: 683–2688, 1997
10. Leroy D, Schmid N, Behr JP, Filhol O, Pares S, Garini J, Bourgarit J-J, Chambaz EM, Cochet C: Direct identification of a polyamine binding domain on the regulatory subunit of the protein kinase casein kinase 2 by photoaffinity labeling. J Biol Chem 270: 17400–17406, 1995
11. Gatica M, Jacob G, Allende CC, Allende JE: DNA inhibits the catalytic activity of the alpha subunit of protein kinase CK2. Biochemistry 34: 122–127, 1995
12. Bodyreff B, Meggio F, Pinna LA, Issinger O-G: Protein kinase CK2 structure-function relationship: Effects of the β- subunit on reconstitution and activity. Cell Mol Biol Res 40: 391–409, 1994
13. Kusk M, Bendixen C, Duno M, Westergaard O, Thomsen B: Protein kinase CK2 structure-function relationship: effects of the beta subunit on reconstitution and activity. Genetic dissection of intersubunit contacts within protein kinase CK2. J Mol Biol 253: 703–711, 1995
14. Krehan A, Lorenz P, Planacoll M, Pyerin W: Interaction sites between catalytic and regulatory subunits in human protein kinase CK2 holoenzymes as indicated by chemical cross-linking and immunological investigations. Biochemistry 35: 4966–4975, 1996
15. Filhol O, Baudler J, Delphin C, Loue-Mackenbach P, Chambaz EM, Cochet CJ: Casein Kinase II and the tumor suppressor protein P53 associate in a molecular complex that is negatively regulated upon P53 phosphorylation. J Biol Chem 267: 20577–20583, 1992
16. Götz C, Wagner P, Issinger O-G, Montenareh M: $p21^{WAF1/CIP1}$ interacts with protein kinase CK2. Oncogene 13: 392–398, 1996
17. Boldyreff B, Issinger O-G: A-Raf kinase is a new interacting partner of protein kinase CK2 β subunit. FEBS Lett 403: 197–199, 1997
18. Hagemann C, Kalmes A, Wixler V, Schuster T, Rapp UR: The regulatory subunit of protein kinase CK2 is a specific A-Raf activator. FEBS Lett 403: 200–202, 1997
19. Chen M, Li D, Krebs EG, Cooper JA: The casein kinase II β subunit binds to Mos and inhibits Mos activity. Mol Cell Biol 17: 1904–1912, 1997
20. Hériché JK, Lebrin F, Rabilloud T, Leroy D, Chambaz EM, Goldberg Y: Regulation of protein phosphatase 2A by direct interaction with casein kinase 2α. Science 276: 952–955, 1997
21. Cosmelli D, Antonelli M, Allende CC, Allende JE: An inactive mutant of the α subunit of protein kinase CK2 that traps the regulatory CK2α subunit. FEBS Lett 410(2–3): 391–396, 1997
22. Hinrichs MV, Jedlicki A, Tellez R, Pongor S, Gatica M, Allende CC, Allende JE: Activity of recombinant alpha-subunits and beta-subunits of casein kinase II from *Xenopus laevis*. Biochemistry 32: 7310–7316, 1993
23. Teramoto H, Crespo P, Coso O, Igishi T, Xu N, Gutkind JS: The small GTP-binding protein Rho activates c-Jun N-terminal kinases/Stress-activated protein kinases in human kidney 293T cells. J Biol Chem 271: 25731–25734, 1996
24. Coso O, Chiarello M, Yu JC, Teramoto H, Crespo P, Xu N, Miki T, Gutkind S: The small GTP-binding proteins Rac I and Cdc 42 regulate the activity of the JNK/SAPK signaling pathway. Cell 81: 1137–1146, 1995
25. Lin PS, Jenson AB, Cowsert L, Nakai Y, Lin LY, Jin XW, Sundberg JP: Distribution and specific identification of papillomavirus major caspid protein epitopes by immunocytochemistry and epitope scanning of synthetic peptides. J Infect Dis 162: 1263–1269, 1990
26. Jedlicki A, Hinrichs MV, Allende CC, Allende JE: The cDNAs coding for the alpha and beta subunits of *Xenopus laevis* casein kinase II. FEBS Lett 297: 280–284, 1992
27. Cullen BR: Methods of eukaryotic expression technology in the functional analysis of cloned genes. Meth Enzymol 152: 684–704, 1987
28. Bradford MM: A rapid and sensitive method for the quatification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254, 1976
29. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685, 1970
30. Hobohm U, Sander C: A sequence property approach to searching protein databases. J Mol Biol 251: 390–399, 1995
31. Wako H, Blundell TL: Use of amino acid environment-dependent substitution tables and con formational propensities in structure prediction from aligned sequences of homologous proteins. 2. Secondary structures. J Mol Biol 238: 693–708, 1994
32. Rost B, Sander C: Prediction of protein secondary structure at better than 70% accuracy. J Mol Biol 232: 584–599, 1993
33. Zhu Z-Y, Blundell TL: The use of amino acid patterns of classified helices and strands in secondary structure prediction. J Mol Biol 260: 261–276, 1996
34. Bozzetti MP, Massari S, Finelli P, Meggio F, Pinna LA, Boldyreff B, Issinger O-G, Palumbo G, Ciriaco C, Bonaccorsi S, Pimpinelli S: The Ste locus, a component of the parasitic *cry-Ste* system of Drosophila melanogaster, encodes a protein that forms crystals in primary spermatocytes and mimics properties of the β subunit of casein kinase 2. Proc Natl Acad Sci USA (Genetics) 92: 6067–6071, 1995
35. Yuvaniyama J, Denu JM, Dixon JE, Saper MA: Crystal structure of the dual-specificity protein phosphatase vhr. Science 272: 1328–1331, 1996
36. Herkowitz I: Functional inactivation of genes by dominant negative mutations. Nature 329: 219–222, 1987
37. Heller-Harrison RA, Czech MP: Enhanced casein kinase II activity in COS-1 cells upon overexpression of either its catalytic or noncatalytic subunit. J Biol Chem 266: 14435–14439, 1991
38. Lüscher B, Litchfield DW: Biosynthesis of casein kinase II in lymphoid cell lines. Eur J Biochem 220: 521–526, 1994
39. Sicheri F, Moarefi I, Kuriyan J: Crystal structure of the Src family tyrosine kinase Hck. Nature 385: 602–609, 1997
40. Xu WQ, Harrison SC, Eck MJ: Three-dimensional structure of the tyrosine kinase c-Src. Nature 385: 595–602, 1997
41. Bidwai AP, Reed JC, Glover CVC: Coding and disruption of CKB1, the gene encoding the 38-kDa β subunit of *Saccharomyces cerevisiae* Casein Kinase II (CKII). J Biol Chem 270: 10395–10404, 1995