# Eukaryotic translation-initiation factor eIF2β binds to protein kinase CK2: effects on CK2α activity

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eIF2 (eukaryotic translation-initiation factor 2) is a substrate and an interacting partner for CK2 (protein kinase CK2). Co-immunoprecipitation of CK2 with eIF2β has now been observed in HeLa cells, overexpressing haemagglutinin-tagged human recombinant eIF2β. A direct association between $His_6$-tagged human recombinant forms of eIF2β subunit and both the catalytic (CK2α) and the regulatory (CK2β) subunits of CK2 has also been shown by using different techniques. Surface plasmon resonance analysis indicated a high affinity in the interaction between eIF2β and CK2α, whereas the affinity for the association with CK2β is much lower. Free CK2α is unable to phosphorylate eIF2β, whereas up to 1.2 mol of phosphate/mol of eIF2β was incorporated by the reconstituted CK2 holoenzyme. The N-terminal third part of eIF2β is dispensable for binding to either CK2α or CK2β, although it contains the phosphorylation sites for CK2. The remaining central/C-terminal part of eIF2β is not phosphorylated by CK2, but is sufficient for binding to both CK2 subunits. The presence of eIF2β inhibited CK2α activity on calmodulin and β-casein, but it had a minor effect on that of the reconstituted CK2 holoenzyme. The truncated forms corresponding to the N-terminal or central/C-terminal regions of eIF2β were much less inhibitory than the intact subunit. The results demonstrate that the ability to associate with CK2 subunits and to serve as a CK2 substrate are confined to different regions in eIF2β and that it may act as an inhibitor on CK2α.

Key words: eukaryotic translation-initiation factor 2 (eIF2), enzymic regulation, protein kinase CK2, protein phosphorylation, protein–protein interaction, surface plasmon resonance.

## INTRODUCTION

Initiation of protein synthesis in eukaryotes requires the participation of multiple protein components, collectively known as eIFs (eukaryotic translation-initiation factors), which interact with the 40 S ribosomal subunit [1,2]. The sequence of intervention of these components is fixed in a precise, clockwise manner. Certain eIFs are believed to participate at various stages of translation initiation, but this seems to be owing to the presence of different domains in their structure, where each one is involved in specific functions. This is exemplified by eIF2, a factor known to play a key role in the regulation of protein synthesis initiation in response to different stimuli. The conventional form of eIF2 is a trimer made of one copy of each of the three different subunits (eIF2α, eIF2β and eIF2γ). Each one of the three subunits contributes to confer the different properties of eIF2, such as GTP binding and GTPase activity, binding to methionyl-initiator tRNA (Met-$tRNA_i^{Met}$), to 40 S ribosomal subunits and to mRNA. Of the three subunits, eIF2β binds mRNA [3] and contributes, together with eIF2γ, to GTP and Met-$tRNA_i^{Met}$ binding [4,5]. A comparative study of the sequences of eukaryal and archaeal eIF2 indicated that the region corresponding to the central and C-terminal domains of eIF2β would be sufficient for the formation of the 43 S preinitiation complex in eukaryotes [4,6], whereas the N-terminal domain would be involved in subsequent steps of translation initiation. In the assembled preinitiation complex, eIF2β remains exposed to the environment, which allows its interaction with other translation factors. The eIF2β mediates the association of eIF2 with eIF2B (which catalyses the GDP to GTP exchange) [7], as well as with eIF5 (which acts as a GTPase-activating protein) [8]. Recently, eIF2β has been shown to interact directly with the adapter protein Nck-1, whose translocation to ribosomes is enhanced by insulin and results in increased protein translation [9].

Translation initiation is well known to be controlled by different extracellular signals. Phosphorylation of eIF2α on $Ser^{51}$ is widely accepted as a main point for this control [1,2], whereas the possible participation of the other eIF2 subunits is less clear. However, it has also been known, for more than two decades, that eIF2β is also phosphorylated *in vivo* [10] and that its phosphate content varies in response to different stimuli [11,12]. Protein sequence analysis of the sites responsible for basal phosphorylation *in vivo* allowed us to propose CK2 (protein kinase CK2 also known as casein kinase 2 or II) as the enzyme responsible for this phosphorylation [13]. CK2 is an enzyme known at present to phosphorylate more than 300 different protein substrates [14]. These also include many protein translation factors, ribosomal proteins and mRNA-binding proteins. Interestingly, both eIF2B (eIF2Bε subunit) [15] and eIF5 [16] have recently been shown to be CK2 substrates *in vivo*. This fact, together with the evidences that considerable levels of CK2 are present in ribosomal-enriched cellular subfractions, suggest that CK2 might be important for maintaining some components of the machinery for protein translation in a functional conformation.

CK2 is accepted as essential for life in eukaryotes, and both the catalytic and the interactive functions of the enzyme seem

Abbreviations used: CK2, protein kinase CK2 (also known as casein kinase 2 or II); Ct, C-terminus; DRB, 5,6-dichlorobenzimidazole riboside; eIFs, eukaryotic translation-initiation factors; HA, haemagglutinin; HS1, haematopoietic lineage cell-specific protein 1; Ni-NTA, $Ni^{2+}$-nitrilotriacetate; Nt, N-terminus; SPR, surface plasmon resonance.

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to be necessary for the progression of the cell cycle [17]. There is a wide array of proteins capable of interacting with either the catalytic (CK2$\alpha$ and CK2$\alpha'$) or the regulatory (CK2$\beta$) subunits of CK2, which may serve to locate CK2 in specific areas of the cell. Many of these proteins are capable of serving as substrates for CK2 and a restricted number of them also exert a regulatory role on CK2 activity.

We had previously reported that a protein of an apparent $M_r$ of 49 000 in SDS/PAGE, associated with CK2 in rat liver, was later shown to correspond to a mixture of eIF2$\beta$ and eIF2$\gamma$ subunits [18]. In the present study, we show that eIF2$\beta$ readily interacts with the catalytic subunit of CK2. This indicates that eIF2$\beta$ may serve to locate CK2 in the protein translation preinitiation complex.

## EXPERIMENTAL

### Reagents and antibodies

Apigenin, DRB (5,6-dichlorobenzimidazole riboside), emodin, polylysine and $\beta$-casein were from Sigma. Calmodulin, LY294002 and spermidine were from Calbiochem. [$\gamma$-$^{32}$P]GTP and [$\gamma$-$^{32}$P]ATP were from Amersham Biosciences. Polyclonal antibodies against CK2$\alpha$, CK2$\beta$ and eIF2$\beta$ were raised by immunization of rabbits with recombinant protein, and the immunoglobulin fraction was obtained from sera by Protein A–agarose chromatography (Amersham Biosciences). Anti-HA (haemagglutinin) (12CA5) antibody was from Roche. Goat anti-rabbit IgG (H + L) alkaline phosphatase or peroxidase-conjugated antibodies were from Bio-Rad (Hercules, CA, U.S.A.).

### Plasmids, protein expression and purification

The catalytic (CK2$\alpha$) and regulatory (CK2$\beta$) subunits of human CK2 were cloned, expressed in *Escherichia coli* and purified as described previously [19]. The cDNA encoding the full-length human eIF2$\beta$ was obtained by reverse transcriptase–PCR using a HeLa cDNA library (Matchmaker cDNA library; ClonTech, Palo Alto, CA, U.S.A.) as a template. N- and C-terminal truncated forms were obtained by PCR from the full-length form. The cDNAs were cloned into pQE-30 vector (Qiagen), expressed in *E. coli* (SG13009) as fusion proteins with a His$_6$ tag in the N-terminal domain and purified by Ni-NTA ($Ni^{2+}$-nitrilotriacetate)–agarose (Qiagen) according to the manufacturer's instructions. pCMV-HA-eIF2$\beta$ mammalian expression vectors were made by PCR amplification from eIF2$\beta$ cDNA and then subcloned. In all cases, sequences were confirmed by DNA sequencing.

### Cell culture and transfection

HeLa and HEK-293 cells were grown on 60 mm diameter plastic Petri dishes in Dulbecco's modified Eagle's medium (Life Technologies, Rockville, MD, U.S.A.) with 4.5 g/l of glucose (Biological Industries, Rockville, MD, U.S.A.) supplemented with 10 % (v/v) foetal bovine serum. Incubation was at 37 °C in a humidified atmosphere containing 5 % $CO_2$. Cells were lysed for 15 min on ice with extraction buffer [100 mM Tris/HCl, pH 7.5/50 mM $\beta$-glycerolphosphate/0.5 mM sodium orthovanadate/1.5 mM EGTA/1 % (v/v) Triton X-100/1 mM PMSF/1 $\mu$g/ml of each leupeptin, pepstatin, benzamidine and aprotinin] for phosphorylation experiments. Alternatively, the cells were lysed with buffer A (50 mM Tris/HCl, pH 7.5/0.4 M NaCl/1.5 mM $MgCl_2$/1.5 mM EGTA/1 mM EDTA/25 mM NaF/0.2 mM $Na_3VO_4$/2 mM $PP_i$/1 mM dithiothreitol/1 % Triton X-100/1 mM PMSF/1 $\mu$g/ml of each leupeptin, pepstatin, benzamidine and aprotinin) for pull-down experiments or with buffer B (50 mM Tris, pH 7.5/100 mM NaCl, 0.1 % Nonidet P40/1 mM PMSF/1 $\mu$g/ml of each leupeptin, pepstatin, benzamidine and aprotinin) for immunoprecipitation experiments. Lysates were centrifuged at 12 000 ***g*** for 30 min at 4 °C in an Eppendorf microfuge and supernatants used for the assays. For cell transfection, cells were seeded at 0.2 × $10^6$ and the next day transfected with LIPOFECTAMINE™ Plus according to the manufacturer's instructions. After 3 h, the medium was re-established overnight and cells were lysed as described above.

### Immunoprecipitation

Protein G–Sepharose (5 $\mu$l), equilibrated with immunoprecipitation buffer, was incubated with 5 $\mu$g of anti-HA antibody. HeLa cell extract protein (500 $\mu$g) was added and incubated for 2 h at 4 °C with gentle shaking. The immunocomplexes were washed twice with immunoprecipitation buffer and once with immunoprecipitation buffer plus 0.5 M NaCl. Proteins attached to the beads were eluted with SDS/PAGE sample buffer subjected to SDS/PAGE, transferred on to PVDF membranes and probed using anti-CK2$\alpha$ antibody.

### Pull-down assays

Human recombinant His$_6$–CK2$\alpha$, His$_6$–CK2$\beta$ or His$_6$–CK2 holoenzyme (4 $\mu$g, reconstituted by mixing His$_6$–CK2$\alpha$ and His$_6$–CK2$\beta$ at a 1:1 molar ratio) was mixed with 250 $\mu$g of HeLa or HEK-293 cellular extracts, previously precleared with Ni-NTA–agarose (Qiagen), for 1 h at 4 °C in lysis buffer. A slurry of Ni-NTA–agarose (1:1; 20 $\mu$l) was added and left for 1 h at 4 °C under gentle shaking. Samples were centrifuged at 12 000 ***g*** for 5 min at 4 °C in an Eppendorf microfuge to sediment the His$_6$-tagged proteins/Ni-NTA–agarose complexes. Beads were washed with lysis buffer A three times and proteins were eluted with SDS/PAGE sample buffer and subjected to SDS/PAGE, transferred on to PVDF membranes and probed using anti-eIF2$\beta$ antibody.

### Far-Western assays

Human recombinant His$_6$–CK2$\alpha$, His$_6$–CK2$\beta$ or His$_6$–eIF2$\beta$ (75 pmol each) were resolved on SDS/PAGE [12 % (w/v) gel], transferred on to PVDF membranes and blocked for 1 h with 5 % dry milk in TPBS buffer [0.1 % (v/v) Tween 20 phosphate-buffered saline (137 mM NaCl/2.7 mM KCl/10 mM $Na_2HPO_4$/1.8 mM $KH_2PO_4$)]. After overnight incubation with renaturalization buffer (50 mM Tris/HCl pH 7.5/300 mM KCl/0.1 % Triton X-100), ligand (20 $\mu$g/ml) or TPBS as a control were added for 5 h with gentle shaking. Membranes were washed three times with TPBS and developed with the indicated antibodies.

### SPR (surface plasmon resonance) analysis

BIAcoreX system (BIAcore, Neuchätel, Switzerland) was used to detect and determine the kinetic constants of the interactions. eIF2$\beta$ molecules were covalently linked to a sensor chip CM5 (BIAcore) using amine coupling chemistry. CK2$\alpha$ and CK2$\beta$ were injected over the surface with a flow rate of 10 $\mu$l/min in running HBS buffer [Hepes-buffered saline (10 mM Hepes,

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pH 7.4/150 mM NaCl/3 mM EDTA/0.005 % (v/v) surfactant P20)] at 25 °C. After injection, HBS replaced the protein solutions in a continuous flow rate of 10 $\mu$l/min. All samples were run simultaneously over a flow cell containing a blank surface. The kinetic data were calculated using the SPR kinetic evaluation software BIAevaluation 3.0 (BIAcore).

### Protein and phosphorylation assays

Protein concentration in the samples was determined by the Bradford method [20] using BSA as standard. For kinase assay using HeLa cell extracts, 5 $\mu$g of sample was left untreated or incubated with the different agents for 5 min at 30 °C in kinase buffer [50 mM Tris/HCl, pH 7.5/1.5 mM EGTA/1.5 mM EDTA/25 mM $MgCl_2$/25 mM $\beta$-glycerolphosphate/1 mM dithiothreitol]. Then 1 $\mu$g of $His_6$–eIF2$\beta$ and 100 $\mu$M [$\gamma$-$^{32}$P]GTP were added as substrates, and samples were incubated further for 30 min at 30 °C. The reactions were stopped by adding SDS/PAGE sample buffer and boiling followed by SDS/PAGE and autoradiography. Radiolabelled bands were detected in a PhosphoImager (Molecular Imager System GS-525; Bio-Rad), excised from the gel and counted in a scintillation counter. Phosphorylation of $His_6$–eIF2$\beta$ forms, $\beta$-casein or calmodulin (0.5 $\mu$g each) *in vitro* was performed at 30 °C for 30 min or 1 h in 25 $\mu$l of a medium containing kinase buffer, 2–5 pmol of $His_6$–CK2 and either 100 $\mu$M [$\gamma$-$^{32}$P]GTP or 100 $\mu$M [$\gamma$-$^{32}$P]-ATP, as indicated. Reactions were stopped and radioactivity analysed as described above. Acid precipitation method was used to determine stoichiometry. Briefly, samples were phosphorylated as described above and 20 $\mu$l was spotted over 2 cm × 2 cm squares of 3MM paper (Whatman), which were soaked in 10 % (w/v) trichloroacetic acid to precipitate the proteins. The paper squares were washed three times in 10 % trichloroacetic acid, dehydrated with ethanol and diethyl ether and then counted in a scintillation counter.

## RESULTS

### Association of CK2 subunits with eIF2

Previous studies have shown that eIF2 and CK2 co-purified through different chromatographic steps and were present in immunocomplexes [21]. The potential role of eIF2$\beta$ in the interaction with CK2 *in vivo* was initially studied combining the expression in HeLa cells of HA-tagged human recombinant eIF2$\beta$ and immunoprecipitation. The results confirmed that a fraction of CK2 catalytic subunits was associated with complexes containing HA–eIF2$\beta$ (Figure 1A). However, this approach is not suitable if the interaction between eIF2$\beta$ and CK2 catalytic subunits was either direct, through other eIF2 subunits, or mediated by other cellular proteins.

We had previously shown that total eIF2$\beta$ levels in different rat tissues were well above those of CK2$\alpha$ and CK2$\beta$ [21], and high levels of eIF2$\beta$ have also been observed in HeLa and HepG2 cell extracts (results not shown). This suggested that a considerable amount of cellular eIF2 must be present in a CK2-free form, which could be used as a source of native eIF2. Thus it was of interest to test if the addition of the purified human recombinant CK2 subunits lead to their association with native eIF2 present in extracts. For this purpose, pull-down experiments were performed as indicated in the Experimental section. The results obtained (Figure 1B) demonstrate that $His_6$–CK2$\alpha$ was able to pull-down eIF2$\beta$ from HeLa and HEK-293 cell extracts. In contrast with this, little eIF2$\beta$, if any, was pulled down by $His_6$–CK2$\beta$. Furthermore,

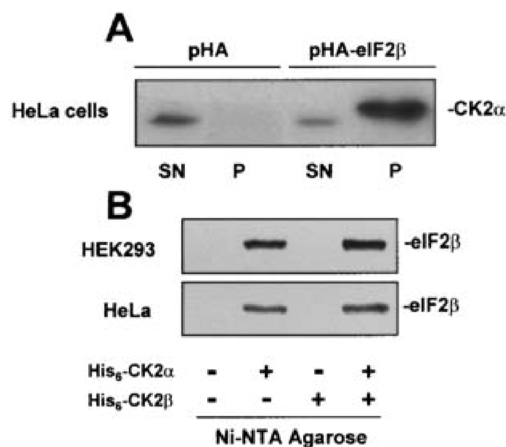


**Figure 1 eIF2$\beta$ interacts with protein kinase CK2 in HeLa cells**

(**A**) pCMV-HA (empty vector) and pCMV-HA-eIF2$\beta$ (encoding full-length eIF2$\beta$) transfected HeLa cells were lysed and a sample (500 $\mu$g of cell extract protein, in a volume of 250 $\mu$l) was immunoprecipitated with anti-HA antibody as described in the Experimental section. The pellet was resuspended in 25 $\mu$l of Laemmli sample buffer and both the supernatants and the pellets were analysed by SDS/PAGE and Western-blot analysis with anti-CK2$\alpha$ antibody. (**B**) Free $His_6$–CK2$\alpha$, $His_6$–CK2$\beta$ or a 1:1 molar mixture of both ($His_6$–CK2$\alpha/\beta$ or reconstituted CK2 holoenzyme) were incubated with 250 $\mu$g of total protein from either HEK-293 or HeLa cell extracts. A slurry of Ni-NTA–agarose (1:1; 20 $\mu$l) was added to recover recombinant proteins and eIF2$\beta$ bound to CK2 subunits was analysed by SDS/PAGE and Western-blot analysis with anti-eIF2$\beta$ antibody.

the reconstituted $His_6$–CK2 holoenzyme, made up by mixing equimolar amounts of $His_6$–CK2$\alpha$ and $His_6$–CK2$\beta$, was able to pull-down eIF2$\beta$. This indicates that the association of CK2$\alpha$ with CK2$\beta$ does not hinder its interaction with eIF2$\beta$.

### Physical interaction between recombinant CK2$\alpha$, CK2$\beta$ and eIF2$\beta$

The use of purified human recombinant forms of eIF2$\beta$, CK2$\alpha$ and CK2$\beta$ allowed us to study their direct physical interaction. Far-Western analysis was used as a first approach. As expected, immobilized CK2$\alpha$ and CK2$\beta$ were capable of interacting with dissolved CK2$\beta$ and CK2$\alpha$ respectively. Positive interaction was also detected between immobilized eIF2$\beta$ and overlaid CK2$\alpha$, as well as between immobilized CK2$\alpha$ and overlaid eIF2$\beta$ (Figure 2). Our analysis also revealed that CK2$\beta$ is capable of interacting with eIF2$\beta$.

Plasmon-resonance analysis has the advantage of allowing the determination of the kinetic constants for the association between interacting molecules. The use of this technique confirmed that both subunits of CK2 were capable of associating with eIF2$\beta$ (Figure 3A). However, the association and dissociation rate constants for the interaction between eIF2$\beta$ and each one of CK2 subunits, calculated from the sensorgrams, were remarkably different (Figure 3B). The results indicate that $His_6$–CK2$\alpha$ associated faster and more efficiently with $His_6$–eIF2$\beta$ than $His_6$–CK2$\beta$. A comparison of the results reported for other interacting partners, such as transcription factor ATF1 (activating transcription factor 1) [22], the HS1 (haematopoietic lineage cell-specific protein 1) [23], the bovine prion protein [24] and grp94 (glucose-regulated protein 94) [25] is also shown in Figure 3(B). Note that the $K_D$ values for the association between $His_6$–CK2$\alpha$ and $His_6$–eIF2$\beta$ are in the same range as those reported for the interaction between CK2$\alpha$ and HS1 [23], but obviously different from that for the interaction between CK2$\alpha$ and CK2$\beta$ [23].

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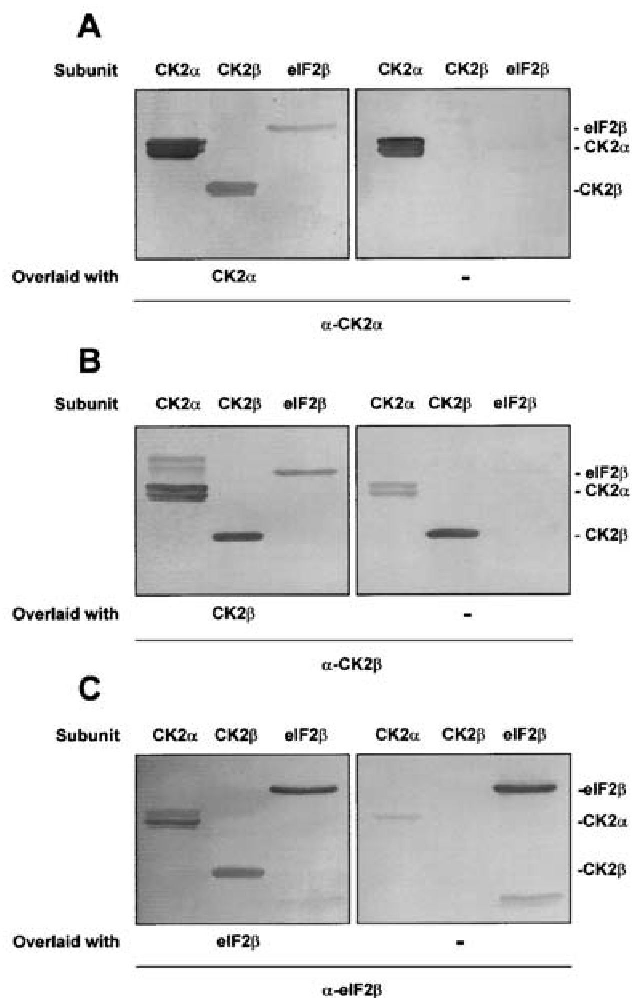
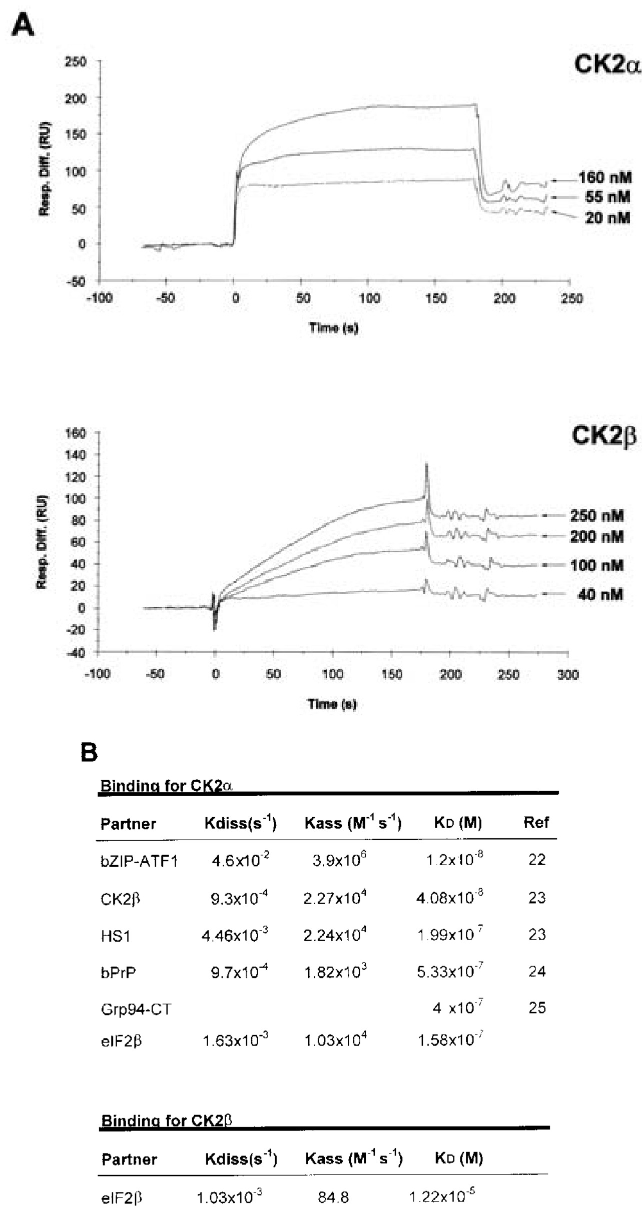



**Figure 2 Analysis of the interaction of $His_6$–eIF2$\beta$ with the catalytic (CK2$\alpha$) and regulatory (CK2$\beta$) subunits of human recombinant CK2 by far-Western assay**

One among $His_6$–CK2$\alpha$ or $His_6$–CK2$\beta$ or $His_6$–eIF2$\beta$ (75 pmol) was resolved on SDS/PAGE (12 % gels) and transferred on to PVDF membranes. Ponceau Red staining was used to confirm transfer efficiency (results not shown). After blocking and renaturalization, the membranes were incubated either in the absence or in the presence of the indicated overlaid protein (20 $\mu$g/ml), washed and developed against (**A**) anti-CK2$\alpha$, (**B**) anti-CK2$\beta$ or (**C**) anti-eIF2$\beta$ antibodies, as indicated.




Binding for CK2α

| Partner | Kdiss($s^{-1}$) | Kass ($M^{-1} s^{-1}$) | KD (M) | Ref |
|---|---|---|---|---|
| bZIP-ATF1 | $4.6\times10^{-2}$ | $3.9\times10^{6}$ | $1.2\times10^{-8}$ | 22 |
| CK2β | $9.3\times10^{-4}$ | $2.27\times10^{4}$ | $4.08\times10^{-8}$ | 23 |
| HS1 | $4.46\times10^{-3}$ | $2.24\times10^{4}$ | $1.99\times10^{-7}$ | 23 |
| bPrP | $9.7\times10^{-4}$ | $1.82\times10^{3}$ | $5.33\times10^{-7}$ | 24 |
| Grp94-CT | | | $4\times10^{-7}$ | 25 |
| eIF2β | $1.63\times10^{-3}$ | $1.03\times10^{4}$ | $1.58\times10^{-7}$ | |

Binding for CK2β

| Partner | Kdiss($s^{-1}$) | Kass ($M^{-1} s^{-1}$) | KD (M) |
|---|---|---|---|
| eIF2β | $1.03\times10^{-3}$ | 84.8 | $1.22\times10^{-5}$ |

**Figure 3 SPR analysis of the interaction of eIF2$\beta$ with the catalytic (CK2$\alpha$) and the regulatory (CK2$\beta$) subunits of human recombinant CK2**

Representative sensorgrams obtained by injection of CK2$\alpha$ or CK2$\beta$ at a flow rate of 10 $\mu$l/ml over a sensor containing immobilized eIF2$\beta$. The response obtained with a control sensor surface (without immobilized protein) was subtracted from each sensorgram. (**A**) Sensorgrams of injected $His_6$–CK2$\alpha$ (upper plot) and $His_6$–CK2$\beta$ (lower plot) at the indicated concentrations. (**B**) Comparison of the estimated kinetic constants for the interactions between CK2 subunits and eIF2$\beta$ with those reported for other partners.

### Phosphorylation of eIF2$\beta$ by human recombinant CK2

It is well established that the regulatory subunit CK2$\beta$ helps the catalytic subunits of CK2 (either CK2$\alpha$ or CK2$\alpha'$) to discriminate between different protein substrates [17]. In this respect, CK2 protein substrates can be grouped into three categories [26]: class I substrates (the majority) are readily phosphorylated by the isolated catalytic subunits and, even better, by the holoenzyme; conversely, phosphorylation of class II substrates (exemplified by calmodulin) is instead prevented by the $\beta$-subunit, which is strictly required for the phosphorylation of class III substrates, exemplified by the Rev protein.

When $His_6$–eIF2$\beta$ was examined as substrate, no phosphorylation was detected with the free $His_6$–CK2$\alpha$, whereas the reconstituted $His_6$–CK2 holoenzyme was clearly active on this substrate (Figure 4A). These results were in agreement with a previous study showing that free recombinant CK2$\alpha$ from *Drosophila* was unable to phosphorylate eIF2$\beta$ within the eIF2 trimer [27], whereas the CK2 holoenzyme, purified from rabbit reticulocytes phosphorylated it. It is worth noting that phosphorylation of $His_6$–eIF2$\beta$ by the reconstituted CK2 holoenzyme resembles that of $\beta$-casein in that it is unaffected by polylysine, but differs in its ability to serve as substrate for free CK2$\alpha$ (Figures 4A and 4B). As shown in Figure 4(B), free $His_6$–CK2$\alpha$ and the holoenzyme behaved as expected from previous reports [28] concerning $\beta$-casein and calmodulin phosphorylation. Taken together, these results indicate that although CK2$\alpha$ is capable

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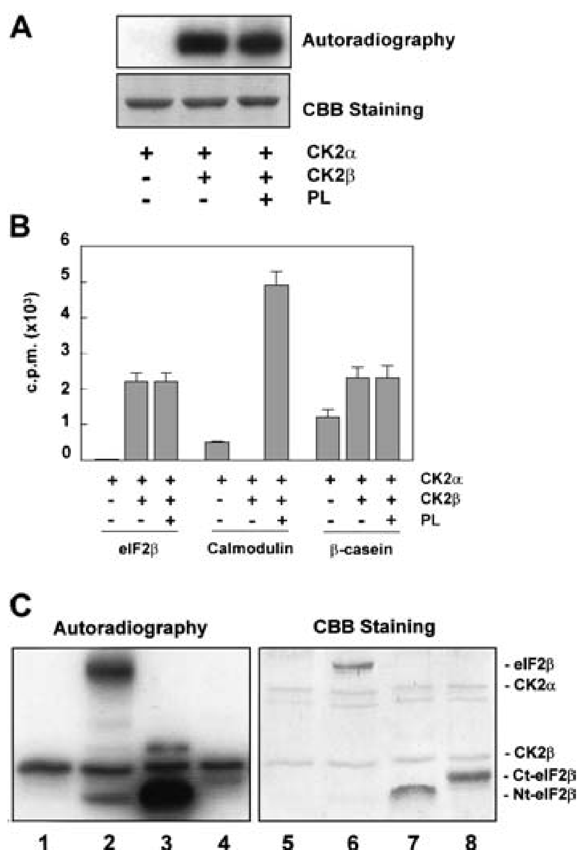


**Figure 4 eIF2$\beta$ phosphorylation by CK2 *in vitro***

(**A**) CK2$\alpha$ (2 pmol) either alone, or in the presence of CK2$\beta$ (at 1:1 molar ratio, to reconstitute CK2 holoenzyme) or with CK2$\beta$ plus polylysine (PL) was used to phosphorylate eIF2$\beta$ at 30 °C for 30 min as described in the Experimental section. Samples were analysed by autoradiography and by Coomassie Brilliant Blue staining (CBB staining). (**B**) Densitometric analysis of eIF2$\beta$, calmodulin and $\beta$-casein phosphorylation by CK2$\alpha$ alone, or with CK2$\beta$ or with CK2$\beta$ and PL. (**C**) CK2 holoenzyme (5 pmol), reconstituted as in (**A**), was used to phosphorylate eIF2$\beta$, Nt-eIF2$\beta$ or Ct-eIF2$\beta$ at 30 °C for 1 h as described in the Experimental section. Samples were analysed by autoradiography and by CBB staining.

of interacting with eIF2$\beta$, the presence of CK2$\beta$ is absolutely required for eIF2$\beta$ phosphorylation. This would assign eIF2$\beta$ to class III CK2 substrates as described in a previous classification [26].

Kinetic parameters for the $His_6$–CK2 holoenzyme were calculated using different concentrations of $His_6$–eIF2$\beta$ (results not shown). A $K_m$ value of $0.49 \pm 0.04$ $\mu$M was estimated from these data. On the other hand, a stoichiometry of $1.25 \pm 0.17$ mol of phosphate/mol of $His_6$–eIF2$\beta$ was estimated in separate experiments. This indicates that $His_6$–eIF2$\beta$ is a good substrate for CK2 holoenzyme, and that it must contain at least two phosphorylation sites in its polypeptide chain. This agrees with previous reports showing the existence of two CK2 phosphorylation sites *in vitro* on the $\beta$-subunit of eIF2 isolated from rabbit reticulocytes [13].

Structural analysis of eIF2$\beta$ revealed the presence of an N-terminal extension that is absent in its homologue from archea [6] (Figure 5A). In the rabbit, this N-terminal region of eIF2$\beta$ nests $Ser^2$ and $Ser^{67}$, identified as two CK2 phosphorylation sites. These two serine residues are conserved in the human eIF2$\beta$ sequence (Figure 5B). Thus we addressed the question of the potential presence of other phosphorylation sites for CK2 in the C-terminal region of eIF2$\beta$, the most conserved part of this protein. To test this hypothesis, we decided to prepare two truncated forms

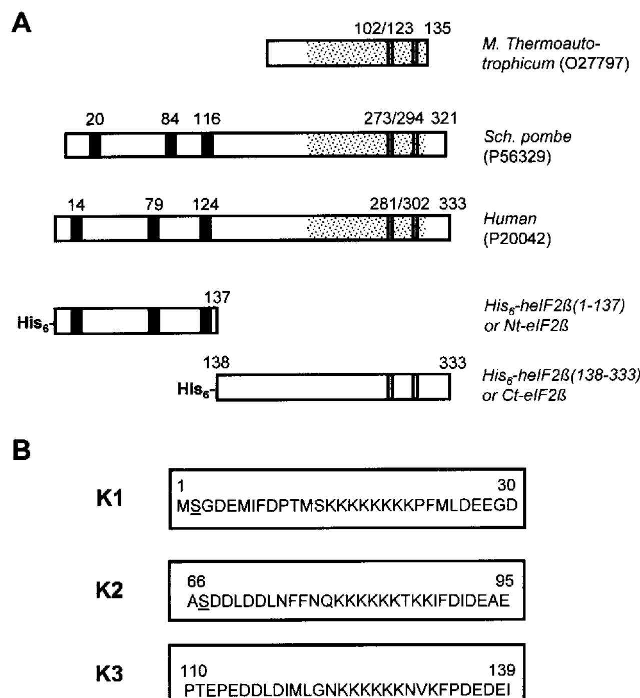


**Figure 5 Structural characteristics of human eIF2$\beta$**

(**A**) Diagrammatical comparison of the structural elements present in the $\beta$-subunit of translation-initiation factor-2. Dotted areas indicate the regions with high amino acid sequence homology between different species, two vertical bars denote the presence of two cysteine residues that participate in $C_2$–$C_2$ finger motifs, and the dark regions indicate the presence of the polylysine. Figures above each diagram denote the amino acid position in the primary structure of each polypeptide. Only the first residue is indicated for the PL stretches and the cysteine pairs. The parentheses contain the accession code numbers for these polypeptides in the SWISS-PROT data bank. (**B**) Amino acid sequence surrounding the PL stretches (K1, K2 and K3) present in human eIF2$\beta$. The position of the reported sites for endogenous phosphorylation by CK2 is underlined.

of human eIF2$\beta$ corresponding to the sequences encompassing residues 1–137 (Nt-eIF2$\beta$, where Nt stands for N-terminus) and 138–333 (Ct-eIF2$\beta$, where Ct stands for C-terminus) respectively preceded by a $His_6$ tag (Figure 5A).

The purified Nt-eIF2$\beta$ and Ct-eIF2$\beta$ behaved as bands of 19 and 23 kDa respectively on SDS/PAGE. Direct N-terminal sequencing confirmed that Nt-eIF2$\beta$ contained a $His_6$ tag followed by the N-terminus of eIF2$\beta$ (residues 1–5 of eIF2$\beta$ were confirmed), whereas Ct-eIF2$\beta$ contained the $His_6$ tag followed by eIF2$\beta$ starting at residue 138 (residues 138–143 were confirmed).

The ability of both truncated forms to serve as substrates for a CK2 holoenzyme was then checked. As expected from previous reports describing $Ser^2$ and $Ser^{67}$ as the main CK2 phosphorylation sites in eIF2$\beta$ [13], Nt-eIF2$\beta$ was phosphorylated by CK2, whereas Ct-eIF2$\beta$ essentially was not, with only a faint band detected after long-term incubation (Figure 4C). Phosphorylation of Nt-eIF2$\beta$ reached a stoichiometry of $1.13 \pm 0.09$ mol of phosphate/mol of polypeptide, and showed a $K_m$ value of $0.45 \pm 0.06$ $\mu$M, values comparable with those observed for full-length $His_6$–eIF2$\beta$.

## Phosphorylation of human recombinant eIF2$\beta$ in crude extracts

The studies reported above were performed using free recombinant subunits or holoenzyme reconstituted *in vitro*. To see if this could reflect the phosphorylation achieved by the native CK2,

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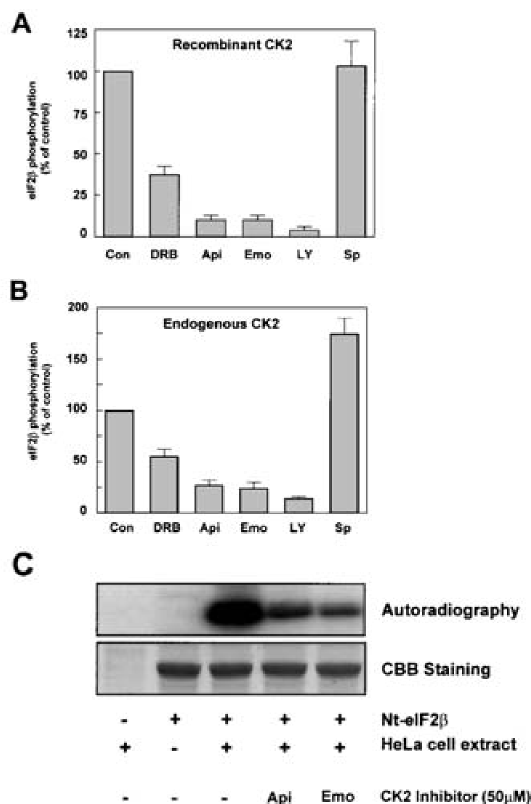


**Figure 6 CK2 inhibitors block eIF2$\beta$ phosphorylation**

(**A**) CK2 holoenzyme (2 pmol) (reconstituted from a 1:1 molar mixture of His$_6$–CK2$\alpha$ and His$_6$–CK2$\beta$) was mixed with purified His$_6$–eIF2$\beta$ and [$\gamma$-$^{32}$P]GTP and subjected to *in vitro* kinase assay either in the absence or in the presence of 50 $\mu$M DRB, 50 $\mu$M apigenin, 50 $\mu$M emodin, 50 $\mu$M LY294002 or 500 $\mu$M spermidine. (**B**) Crude extracts from asynchronous growing HeLa cells (5 $\mu$g of total protein) were subjected to *in vitro* kinase assay using His$_6$–eIF2$\beta$ as substrate, under conditions similar to those described in (**A**). In both (**A**) and (**B**), the phosphorylation reactions were stopped by the addition of Laemmli sample buffer and the samples were resolved on SDS/PAGE (12 % gel). The radiolabelled eIF2$\beta$ bands were detected by autoradiography and excised from the gels and then counted in a scintillation counter. Means $\pm$ S.D. of the data from three independent experiments are plotted relative to the control without added chemical modulators. (**C**) Crude extracts from asynchronous growing HeLa cells (5 $\mu$g of total protein) were incubated with purified Nt-eIF2$\beta$ in the presence of [$\gamma$-$^{32}$P]GTP under *in vitro* kinase assay conditions either without or with 50 $\mu$M apigenin or 50 $\mu$M emodin. Samples were analysed by SDS/PAGE followed by autoradiography, and by CBB staining.

we decided to compare the effect of a set of chemical modulators of CK2 on His$_6$–eIF2$\beta$ phosphorylation by the reconstituted CK2 and the enzyme present in cell extracts. As can be observed in Figures 6(A) and 6(B), in the presence of the known inhibitors DRB [29], apigenin [30], emodin [30–32] and LY294002 [33], the response of the reconstituted enzyme matched that of the native CK2. On the other hand, the activation of the native CK2 present in the extracts by spermine is probably due to the presence of endogenous inhibitors of CK2 activity in cell extracts whose effect is counteracted by polyamines, a fact known for many years [34,35]. The Nt-eIF2$\beta$ mutant was also efficiently phosphorylated by native CK2 present in HeLa cell extracts in a reaction that was inhibited by apigenin and emodin (Figure 6C).

### Effect of eIF2$\beta$ on CK2 activity in calmodulin and $\beta$-casein

The results reported above showed that free CK2$\alpha$ was capable of associating with eIF2$\beta$, but it was unable to phosphorylate it. This indicated that their association differed from the classical enzyme–substrate interaction, which led us to explore the possible consequences of CK2 activity on other substrates. A different behaviour was observed with free His$_6$–CK2$\alpha$ and the reconstituted His$_6$–CK2 holoenzyme (Figure 7A). The presence of His$_6$–eIF2$\beta$, even at high molar ratios, did not significantly affect the activity of the holoenzyme on $\beta$-casein and had only a moderate inhibitory effect on the phosphorylation of calmodulin (the latter assayed in the presence of polylysine). In contrast, His$_6$–eIF2$\beta$ inhibited His$_6$–CK2$\alpha$ activity on either $\beta$-casein or calmodulin in a His$_6$–eIF2$\beta$ concentration-dependent way, with 50 % inhibition observed at molar ratios of approx. 1:1 for $\beta$-casein and 2:1 for calmodulin.

### Truncation of eIF2$\beta$ impairs its inhibitory effects on CK2 activity

As confirmed by the results reported above, the N-terminal region harbours the CK2 phosphorylation sites present in eIF2$\beta$. Another remarkable feature of this extended polypeptide chain is the presence of three blocks of polylysines, each one flanked by stretches of acidic residues (Figures 5A and 5B). Polybasic amino acid stretches present in other proteins have been shown to affect CK2 activity *in vitro* [36–40]. Hence we studied the possibility that the N-terminal region was also responsible for the modulatory effects exerted on CK2$\alpha$ by intact eIF2$\beta$. Unexpectedly, the results obtained on the phosphorylation of either $\beta$-casein or calmodulin showed that although each one of the truncated forms of eIF2$\beta$ was capable of partially inhibiting CK2$\alpha$ activity, neither of them was as efficient as the intact eIF2$\beta$ (Figure 7B). This indicated that intact eIF2$\beta$ is required for the inhibitory effect on CK2$\alpha$. Control experiments using high molar ratios of either ovalbumin or BSA confirmed that the effect was specific for eIF2$\beta$ and not the mere result of higher total protein content.

### The N-terminal region of eIF2$\beta$ is dispensable for binding to CK2

The results obtained above prompted us to explore the potential need of intact eIF2$\beta$ to bind CK2 subunits. Far-Western analysis was used, since this technique had allowed the easy detection of eIF2$\beta$ binding to the two CK2 subunits. The results (Figure 8) indicated that Ct-eIF2$\beta$ was capable of binding both CK2 subunits, whereas no binding was detected with Nt-eIF2$\beta$. Ponceau Red staining indicated that similar amounts of protein had been blotted to the membrane (results not shown). Taken together, these results on eIF2$\beta$ phosphorylation and binding to CK2 indicated that the ability to serve as substrate and to associate with CK2 resided in different regions of eIF2$\beta$.

## DISCUSSION

Among protein kinases, CK2 is a known enzyme with the unique property of being constitutively active [14,17]. Considerable efforts made by different groups to find a hallmark physiological modulator of this enzyme have given discouraging results. In some cases, the enzyme has been proven to be affected *in vitro* by other cellular components, but reasons based on their cellular distribution, differences between their intracellular concentration and that required to affect CK2, or absence of correlation between CK2 activity and the changes in their concentration in response to stimuli have prevented consideration of their use as authentic physiological modulators. Nevertheless, it is also well documented that the CK2 is crucial for the life of eukaryotic cells, its cellular content is markedly increased on stimulation of cell proliferation and in cancer cells, and that it shuttles between

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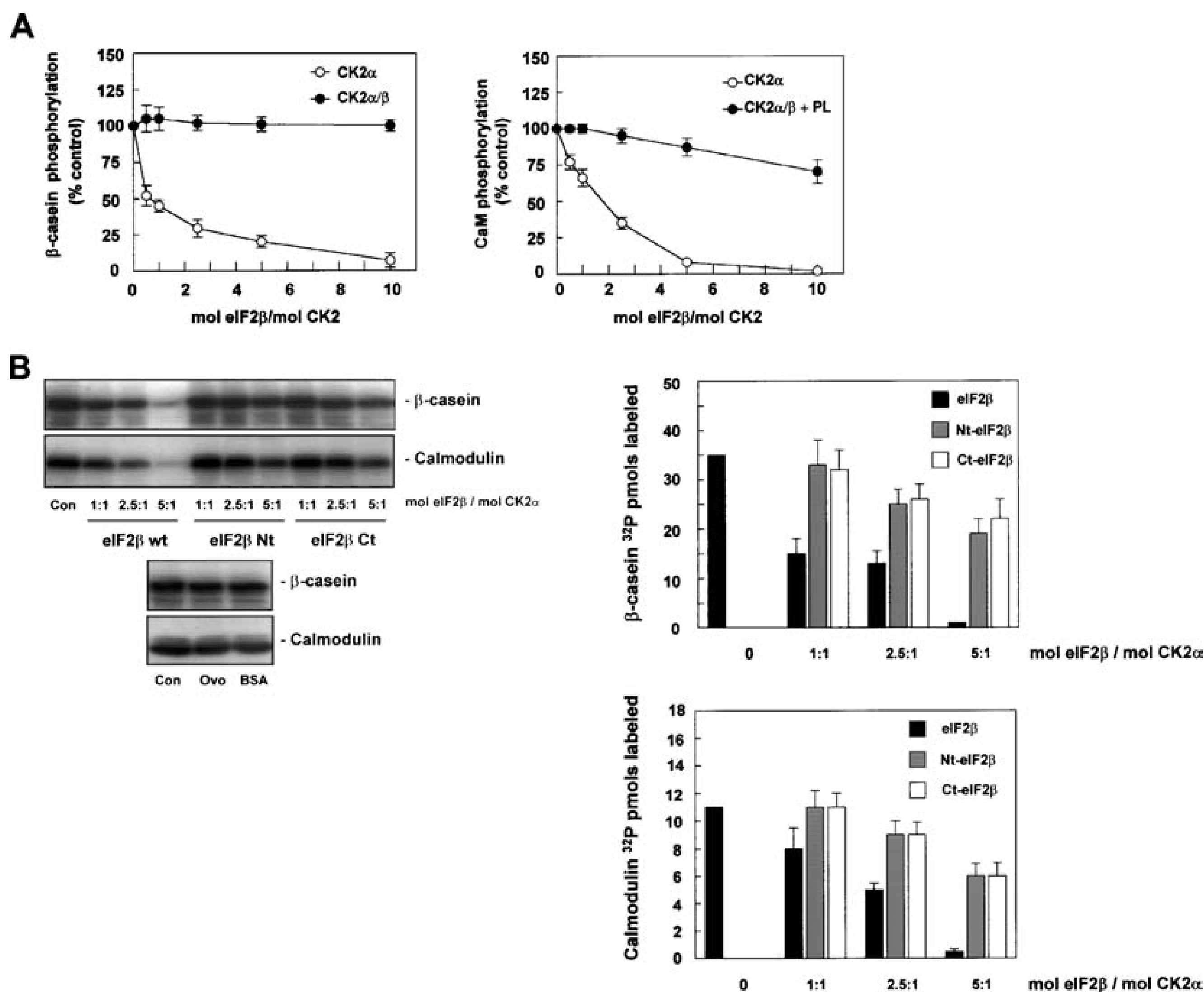


**Figure 7 Modulation of CK2$\alpha$ activity by eIF2$\beta$**

(**A**) Free CK2$\alpha$ or CK2 holoenzyme (reconstituted from a 1:1 molar mixture of His$_6$–CK2$\alpha$ and His$_6$–CK2$\beta$) activities were assayed using either $\beta$-casein (upper panel) or calmodulin (lower panel) as substrate in the presence of increasing amounts of eIF2$\beta$. PL was added to the assays using CK2 holoenzyme and calmodulin as substrate, but not in the other phosphorylation assays. Phosphorylated samples were analysed by SDS/PAGE followed by autoradiography. (**B**) Phosphorylation of either $\beta$-casein or calmodulin by CK2$\alpha$ was performed at 30 °C for 30 min in the presence of increasing amounts of wild-type eIF2$\beta$ or Nt-eIF2$\beta$ or Ct-eIF2$\beta$ (upper panels). Separate phosphorylation experiments, using either ovalbumin (Ovo) or BSA at molar ratios of 20:1 instead of eIF2$\beta$ or its truncated forms, were performed as controls (Con) (lower panels). Phosphorylated samples were analysed by SDS/PAGE followed by autoradiography (upper and lower panels). Then, the radiolabelled bands were excised from the gels and counted in a scintillation counter. The means ± S.D. of the data from three independent experiments are shown in the plots.

cytosol and nucleus in response to different stimuli [17,41]. All this evidence points to the idea that CK2 is probably subject to control mechanisms within living cells.

The most conventional form of CK2 is a tetramer composed of two catalytic (CK2$\alpha/\alpha'$) and two regulatory (CK2$\beta$) subunits. However, there is increasing evidence of the existence of CK2$\alpha/\alpha'$ and CK2$\beta$ not assembled to each other but associated with other cellular proteins [42–44]. In fact, the tetrameric CK2 forms a complex with other cellular proteins [17]. The potential meaning of these interactions was initially considered to serve to locate CK2 in specific cellular regions/processes, but recent results have revealed that they may also contribute to the modulation CK2 activity. In previous work, we observed that eIF2 co-immuno-precipitated from extracts with CK2 [21] and inhibited CK2 activity on casein, a fact that was attributed to the known ability of eIF2$\beta$ to serve as a substrate for CK2. Results from the present study show that the ability to associate with CK2 subunits and to serve as a CK2 substrate are confined to different regions in eIF2$\beta$.

The lack of association with CK2 subunits and the absence of marked effects on CK2 activity observed with the truncated form encompassing the N-terminal third of eIF2$\beta$ was somehow unexpected when considering its structural features. eIF2$\beta$ contains three well-defined basic polylysine stretches, as well as several acidic amino acid stretches, and both basic stretches as well as polyglutamyl peptides are known to be important elements either for the binding of the CK2 subunits to other cellular proteins and/or to modulate CK2 activity [36–40,45]. Furthermore, the N-terminal region contains all the phosphorylation sites for CK2 reported for native eIF2$\beta$ [13] and it is the sole region found to be

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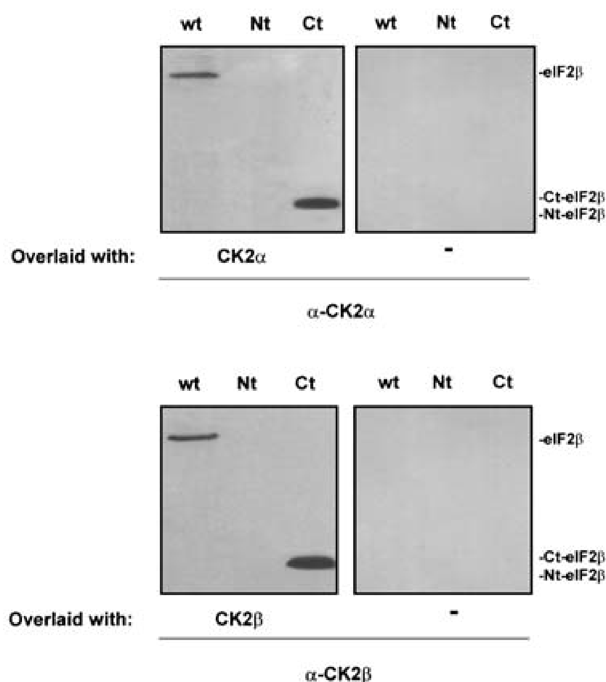


**Figure 8 Far-Western analysis of the interaction between wild-type eIF2$\beta$ or its truncated forms with CK2 subunits**

Wild-type eIF2$\beta$ (wt) or Nt-eIF2$\beta$ (Nt) or Ct-eIF2$\beta$ (Ct) recombinant forms (75 pmol of each) was resolved on SDS/PAGE (15 % gels) and transferred on to PVDF membranes. Ponceau Red staining was used to confirm transfer efficiency (results not shown). After blocking and renaturalization, the membranes were incubated either in the absence or in the presence of the indicated overlaid protein (20 $\mu$g/ml), washed and developed against anti-CK2$\alpha$ or anti-CK2$\beta$ antibodies.

efficiently phosphorylated by CK2 in the present studies. In any case, these results, together with the positive binding of CK2$\alpha$ and CK2$\beta$ to the central/C-terminal form of eIF2$\beta$, devoid of phosphorylation sites for CK2, would indicate strongly that the association between eIF2$\beta$ and CK2 subunits is not merely the result of a typical enzyme–substrate interaction.

When considering eIF2$\beta$, it is generally assumed that its physiological role implies its assembling with the two other eIF2 subunits to form the trimer. However, a recent report has shown that the presence of leptomycin B, an agent that blocks nuclear export, results in a predominant nuclear accumulation of eIF2$\beta$, whereas eIF2$\gamma$ remains almost exclusively cytoplasmic [46]. The potential functions of free eIF2$\beta$, and its role in a nucleus, remain undisclosed. Our present results disclose the possibility that it might contribute to the regulation of CK2$\alpha$. Pertinent to this are the facts that CK2 shows cytosol/nuclear dynamics in response to different stimuli [17,41] and the presence of catalytic subunits in nucleus bound to cellular components other than CK2$\beta$ [42].

Previous studies on CK2 binding to other cellular proteins have shown that their interaction may result in a variety of effects. In some cases, such as the activation caused by highly basic ribosomal protein L41 [39], or the inhibition by peptidyl-prolyl isomerase Pin1 [47], the effects are restricted to a specific protein substrate (DNA topoisomerase II$\alpha$) without having consequences on the phosphorylation of other protein or synthetic peptide substrates. Binding to a partner may affect the activities of both CK2 holoenzyme and free catalytic subunits, as observed with the inhibitory effects caused by the adenomatous polyposis coli protein [48]. In others, the effects are more dramatic on free CK2$\alpha$ than on the holoenzyme, as exemplified by the HS1, which inhibits calmodulin phosphorylation by CK2$\alpha$ [23], and bovine prion protein, which activates it [24]. Interaction with a partner might even have opposite effects on the activities of either CK2$\alpha$ or on the holoenzyme, a good example being provided by the interaction between p53 and CK2. Intact p53, as well as the truncated form corresponding to its C-terminal region, activate mouse double minute 2 phosphorylation by CK2 holoenzyme but inhibit the phosphorylation of this substrate by free CK2$\alpha$ [38]. The effects of eIF2$\beta$ would resemble those of HS1 in that they are mainly exerted inhibiting CK2$\alpha$. However, the association between CK2 and these partners is different in that eIF2$\beta$ also interacts with CK2$\beta$, whereas HS1 does not [23]. Furthermore, HS1 is a good substrate for free CK2$\alpha$ [49], whereas phosphorylation of eIF2$\beta$ is entirely dependent on the presence of CK2$\beta$.

In summary, our present results show that the central/C-terminal region of eIF2$\beta$ is responsible for binding to CK2$\alpha$, which would result in a down-regulation of its kinase activity. The concomitant presence of CK2$\beta$ leads to reconstitution of the CK2 holoenzyme, overcoming the inhibitory effect of eIF2$\beta$ and promoting the phosphorylation of eIF2$\beta$ itself on its N-terminal region. This illustrates how multifarious the association of CK2 with its partners can be and highlights the potential role of eIF2$\beta$ as a modulator of CK2$\alpha$ in addition to its well-known effects on protein synthesis initiation.

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## REFERENCES


1 Kimball, S. R. (1999) Eukaryotic initiation factor eIF2. Int. J. Biochem. Cell Biol. **31**, 25–29

2 Sonenberg, N. and Dever, T. E. (2003) Eukaryotic translation initiation factors and regulators. Curr. Opin. Struct. Biol. **13**, 56–63

3 Laurino, J. P., Thompson, G. M., Pacheco, E. and Castilho, B. A. (1999) The $\beta$ subunit of eukaryotic initiation factor 2 binds mRNA through the lysine repeats and a region comprising the C2–C2 motif. Mol. Cell. Biol. **19**, 173–181

4 Thompson, G. M., Pacheco, E., Melo, E. O. and Castilho, B. A. (2000) Conserved sequences in the $\beta$ subunit of archeal and eukaryal translation initiation factor 2 (eIF2), absent from eIF5, mediate interaction with eIF2$\beta$. Biochem. J. **347**, 703–709

5 Nika, J., Rippel, S. and Hannig, E. M. (2001) Biochemical analysis of the eIF2$\beta\gamma$ complex reveals a structural function for eIF2$\alpha$ in catalyzed nucleotide exchange. J. Biol. Chem. **276**, 1051–1056

6 Cho, S. and Hoffman, D. W. (2002) Structure of the $\beta$ subunit of translation initiation factor 2 from the archaeon *Methanococcus jannaschii*: a representative of the eIF2$\beta$/eIF5 family of proteins. Biochemistry **41**, 5730–5742

7 Kimball, S. R., Heinzinger, N. K., Horetsky, R. L. and Jefferson, L. S. (1998) Identification of interprotein interactions between the subunits of eukaryotic initiation factors eIF2 and eIF2B. J. Biol. Chem. **273**, 3039–3044

8 Das, S., Maiti, T., Das, K. and Maitra, U. (1997) Specific interaction of eukaryotic translation initiation factor 5 (eIF5) with the $\beta$ subunit of eIF2. J. Biol. Chem. **272**, 31712–31718

9 Kebache, S., Zuo, D., Chevet, E. and Larose, L. (2002) Modulation of protein translation by Nck-1. Proc. Natl. Acad. Sci. U.S.A. **99**, 5406–5411

10 Tuazon, P. T., Merrick, W. C. and Traugh, J. A. (1980) Site-specific phosphorylation of initiation factor 2 by three cyclic nucleotide-independent protein kinases. J. Biol. Chem. **255**, 10954–10958

11 Duncan, R. and Hershey, J. W. B. (1985) Regulation of initiation factors during translational repression caused by serum depletion. J. Biol. Chem. **260**, 5493–5497

12 Luis, A. M., Izquierdo, J. M., Ostronoff, L. F., Salinas, M., Santarén, J. F. and Cuezva, J. M. (1993) Translational regulation of mitochondrial differentiation in neonatal rat liver. J. Biol. Chem. **268**, 1868–1875


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13 Welsh, G. I., Price, N. T., Bladergroen, B. B., Bloomberg, G. and Proud, C. G. (1994) Identification of novel phosphorylation sites in the $\beta$-subunit of translation initiation factor eIF-2. Biochem. Biophys. Res. Commun. **201**, 1279–1288
14 Meggio, F. and Pinna, L. A. (2003) One-thousand-and-one substrates of protein kinase CK2? FASEB J. **17**, 349–368
15 Wang, X., Paulin, F. E. M., Campbell, L. E., Gomez, E., O'Brien, K., Morrice, N. and Proud, C. G. (2001) Eukaryotic initiation factor 2B: identification of multiple phosphorylation sites in the $\varepsilon$-subunit and their functions *in vivo*. EMBO J. **20**, 4349–4359
16 Majumdar, R., Bandyopadhyay, A., Deng, H. and Maitra, U. (2002) Phosphorylation of mammalian translation initiation factor 5 (eIF5) *in vitro* and *in vivo*. Nucleic Acids Res. **30**, 1154–1162
17 Litchfield, D. W. (2003) Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochem. J. **369**, 1–15
18 Gil, C., Plana, M., Riera, M. and Itarte, E. (1996) Rat liver pp49, a protein that forms complexes with protein kinase CK2, is composed of the $\beta$ and the $\gamma$ subunits of translation initiation factor eIF-2. Biochem. Biophys. Res. Commun. **225**, 1052–1057
19 Roher, N., Miró, F., Boldyreff, B., Llorens, F., Plana, M., Issinger, O.-G. and Itarte, E. (2001) The C-terminal domain of human grp94 protects the catalytic subunit of protein kinase CK2 (CK2$\alpha$) against thermal aggregation. Role of disulfide bonds. Eur. J. Biochem. **268**, 429–436
20 Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. **72**, 248–254
21 Riera, M., Roher, N., Miró, F., Gil, C., Trujillo, R., Aguilera, J., Plana, M. and Itarte, E. (1999) Association of protein kinase CK2 with eukaryotic translation initiation factor eIF-2 and with grp94/endoplasmin. Mol. Cell. Biochem. **191**, 97–104
22 Yamaguchi, Y., Wada, T., Suzuki, F., Takagi, T., Hasegawa, J. and Handa, H. (1998) Casein kinase II interacts with the bZIP domains of several transcription factors. Nucleic Acids Res. **26**, 3854–3861
23 Ruzzene, M., Brunati, A. M., Sarno, S., Donella-Deana, A. and Pinna, L. A. (1999) Hematopoietic lineage cell specific protein 1 associates with and down-regulates protein kinase CK2. FEBS Lett. **461**, 32–36
24 Meggio, F., Negro, A., Sarno, S., Ruzzene, M., Bertoli, A., Sorgato, M. A. and Pinna, L. (2000) Bovine prion protein as a modulator of protein kinase CK2. Biochem. J. **352**, 191–196
25 Roher, N., Sarno, S., Miró, F., Ruzzene, M., Llorens, F., Meggio, F., Itarte, E., Pinna, L. A. and Plana, M. (2001) The carboxy-terminal domain of Grp94 binds to protein kinase CK2$\alpha$ but not to holoenzyme. FEBS Lett. **505**, 42–46
26 Pinna, L. A. (2002) Protein kinase CK2: a challenge to canons. J. Cell Sci. **115**, 3873–3878
27 Lin, W.-J., Tuazon, P. T. and Traugh, J. A. (1991) Characterization of the catalytic subunit of casein kinase II expressed in *Escherichia coli* and regulation of activity. J. Biol. Chem. **266**, 5664–5669
28 Meggio, F., Boldyreff, B., Marin, O., Marchiori, F., Perich, J. W., Issinger, O.-G. and Pinna, L. A. (1992) The effect of poly-lysine on casein-kinase-2 activity is influenced by both the structure of the protein/peptide substrates and the subunit composition of the enzyme. Eur. J. Biochem. **205**, 939–945
29 Zandomeni, R., Zandomeni, M. C. and Weinmann, R. (1986) Casein kinase type II is involved in the inhibition by 5,6-dichloro-1-$\beta$-D-ribofuranosylbenzimidazole of specific RNA polymerase II transcription. J. Biol. Chem. **261**, 3414–3419
30 Channavajhala, P. and Seldin, D. C. (2002) Functional interaction of protein kinase CK2 and c-Myc in lymphomagenesis. Oncogene **21**, 5280–5288
31 Battistutta, R., Sarno, S., De Moliner, E., Papinutto, E., Zanotti, G. and Pinna, L. A. (2000) The replacement of ATP by the competitive inhibitor emodin induces conformational modifications in the catalytic site of protein kinase CK2. J. Biol. Chem. **275**, 29618–29622
32 Sarno, S., Moro, S., Meggio, F., Zagotto, G., Dal Ben, D., Ghisellini, P., Battistutta, R., Zanotti, G. and Pinna, L. A. (2002) Towards the rational design of protein kinase-2 inhibitors. Pharmacol. Ther. **93**, 159–168
33 Davies, S. P., Reddy, H., Caivano, M. and Cohen, P. (2000) Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem. J. **351**, 95–105
34 Job, D., Pirollet, F., Cochet, C. and Chambaz, E. M. (1979) Interaction of a casein kinase (G-type) with a specific endogenous inhibitor. Possible target for the regulation of a cyclic nucleotide-independent protein kinase activity by polyamines. FEBS Lett. **108**, 508–512
35 Bertomeu, J. F., Guasch, M. D., Plana, M. and Itarte, E. (1981) Rat liver cytosol contains an inhibitor of the casein kinases 1 and 2 from the same source. FEBS Lett. **124**, 261–264
36 Meggio, F., Boldyreff, B., Issinger, O.-G. and Pinna, L. A. (1994) 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
37 Ohtsuki, K., Nishikawa, Y., Saito, H., Munakata, H. and Kato, T. (1996) DNA-binding sperm proteins with oligo-arginine clusters function as potent activators for egg CK-II. FEBS Lett. **378**, 115–120
38 Guerra, B., Gotz, C., Wagner, P., Montenarh, M. and Issinger, O.-G. (1997) The carboxy terminus of p53 mimics the poly-lysine effect of protein kinase CK2-catalysed MDM2 phosphorylation. Oncogene **14**, 2683–2688
39 Lee, J.-H., Kim, J.-M., Kim, M.-S., Lee, Y.-T., Marshak, D. R. and Bae, Y.-S. (1997) The highly basic ribosomal protein L41 interacts with the $\beta$ subunit of protein kinase CKII and stimulates phosphorylation of DNA topoisomerase II$\alpha$ by CKII. Biochem. Biophys. Res. Commun. **238**, 462–467
40 Ohtsuki, K., Maekawa, T., Harada, S., Karino, A., Morikawa, Y. and Ito, M. (1998) Biochemical characterization of HIV-1 Rev as a potent activator of casein kinase II *in vitro*. FEBS Lett. **428**, 235–240
41 Miró, F. A., Llorens, F., Roher, N., Plana, M., Gómez, N. and Itarte, E. (2002) Persistent nuclear accumulation of protein kinase CK2 during the G1-phase of the cell cycle does not depend on the ERK1/2 pathway but requires active protein synthesis. Arch. Biochem. Biophys. **406**, 165–172
42 Stigare, J., Buddelmeijer, N., Pigon, A. and Egyhazi, E. (1993) A majority of casein kinase II $\alpha$ subunit is tightly bound to intranuclear components but not to the $\beta$ subunit. Mol. Cell. Biochem. **129**, 77–85
43 Guerra, B., Siemer, S., Boldyreff, B. and Issinger, O.-G. (1999) Protein kinase CK2: evidence for a protein kinase CK2$\beta$ subunit fraction, devoid of the catalytic CK2$\alpha$ subunit, in mouse brain and testicles. FEBS Lett. **462**, 353–357
44 Guerra, B. and Issinger, O.-G. (1999) Protein kinase CK2 and its role in cellular proliferation, development and pathology. Electrophoresis **20**, 391–408
45 Meggio, F., Pinna, L. A., Marchiori, F. and Borin, G. (1983) Polyglutamyl peptides: a new class of inhibitors of type-2 casein kinases. FEBS Lett. **162**, 235–238
46 Bohnsack, M. T., Regener, K., Schwappach, B., Saffrich, R., Paraskeva, E., Hartmann, E. and Görlich, E. (2002) Exp5 exports eEF1A via tRNA from nuclei and synergizes with other transport pathways to confine translation to the cytoplasm. EMBO J. **21**, 6205–6215
47 Messenger, M. M., Saulnier, R. B., Gilchrist, A. D., Diamond, P., Gorbsky, G. J. and Litchfield, D. W. (2002) Interactions between protein kinase CK2 and Pin1. J. Biol. Chem. **277**, 23054–23064
48 Homma, M. K., Li, D., Krebs, E. G., Yuasa, Y. and Homma, Y. (2002) Association and regulation of casein kinase 2 activity by adenomatous polyposis coli protein. Proc. Natl. Acad. Sci. U.S.A. **99**, 5959–5964
49 Ruzzene, M., Brunati, A. M., Sarno, S., Marin, O., Donella-Deana, A. and Pinna, L. A. (2000) Ser/Thr phosphorylation of hematopoietic specific protein 1 (HS1). Eur. J. Biochem. **267**, 3065–3072

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