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Yeast Elf1 Factor Is Phosphorylated and Interacts with Protein Kinase CK2

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One of the biggest group of proteins influenced by protein kinase CK2 is formed by factors engaged in gene expression. Here we have reported recently identified yeast transcription elongation factor Elf1 as a new substrate for both monomeric and tetrameric forms of CK2. Elf1 serves as a substrate for both the recombinant CK2α' (K_m 0.38 μM) and holoenzyme (K_m 0.13 μM). By MALDI-MS we identified the two serine residues at positions 95 and 117 as the most probable *in vitro* phosphorylation sites. Co-immunoprecypitation experiments show that Elf1 interacts with catalytic (α and α') as well as regulatory (β and β') subunits of CK2. These data may help to elucidate the role of protein kinase CK2 and Elf1 in the regulation of transcription elongation.

Keywords: Cloning and overexpression, Mass spectrometry, Phosphorylation, Protein-protein interaction, Protein kinase CK2, Transcription, Transcription factor Elf1, Yeast

Introduction

Research data of the past decades indicate that transcription factors play a critical role in many biological events. The activities of individual transcription effectors can be regulated at various levels including post-translational modification and intracellular translocation. Reversible protein phosphorylation has been established as a crucial regulatory mechanism of the transcription factors regulation. By means of this mechanism the activity of many transcription factors is regulated both positively and negatively (Hunter, 2000).

Addition or removal one or more phosphate groups on Ser, Thr or Tyr residues by protein kinase or protein phosphatase, respectively, can affect protein activity. The negative charge introduced by a phosphorylated amino acid residue can modify the activity of protein by induction of allosteric conformational changes, as well as by attracting and repulsive forces (Hurley *et al.*, 1990; Whitmarsh and Davis, 2000). The cyclic AMP response element-binding protein (CREB), was the first transcription factor shown to be regulated by reversible phosphorylation (Gonzales and Montminy, 1989). This ubiquitous regulatory factor binds to the cAMP response element (CRE) and stimulates transcription after phosphorylation on Ser^{133} by PKA (Mayr and Montminy, 2001). Such phosphorylation based regulatory mechanism is not restricted only to the CREB, but includes many others such as activating transcription factor 2 (ATF2), p53, HSF1, STAT, NFAT, Gcn4, E2F1, AP1, STATs, TCF, β-catenin and many others (Holmberg *et al.*, 2002).

Phosphorylation directly modulates the activity of transcription factors by different ways. It may influence stability of the protein by protection against their degradation as it is in case of ATF2 (Fuchs *et al.*, 2000) or is required for their proteolytic degradation as observed in case of MyoD (Song *et al.*, 1999) and E2F (Vandel and Kouzarides, 1999). In some cases phosphorylation modifies cellular localization. For example phosphorylation changes the conformation of the regulatory domain of NFAT from a flexible globular ensemble to a rigid helical bundle, blocking access to the nuclear localization sequence (Shen *et al.*, 2005). It has been shown that phosphorylation affects the DNA-binding activity. Phosphorylation of the transcription factor STAT promotes its DNA-binding activity (Kisseleva *et al.*, 2002) when phosphate-modification of c-Jun is both positively and negatively regulated in a phosphorylation site-specific manner (Dunn *et al.*, 2002). Phosphorylation of CREB facilitates its association with the co-activator, CREB-binding protein (CBP) (Wagner *et al.*, 2002), while phosphorylation of the STAT transcription factor affects its dimerization (Whitmarsh and Davis, 2000). The

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transcriptional activity of a major stress protein transcription factor, heat shock factor (HSF1), is also regulated by reversible phosphorylation (Holmberg *et al.*, 2002; Hashikawa and Sakurai, 2004; Conde *et al.*, 2005).

The human genome encodes more than thousand protein kinases phosphorylating as much as 30% of cellular proteins (Hunter, 2000). In eucaryotic organisms, two broad classes of protein kinases are well described: Tyr protein kinases and Ser/Thr protein kinases. Generally, first group responds to growth factors and mitogenic signals to initiate quick signal transduction, while Ser/Thr kinases are rather responsible for integration and amplification of signals. In a number of cases, Ser/Thr protein kinases regulate signalling cascades at the level of transcription factors and gene expression.

Protein kinase CK2 is a holoenzyme belonging to the big family of Ser/Thr kinases. It consists of two catalytic (α and α') associated with the dimmer of regulatory subunits (β). Distinct isozymes of the CK2 catalytic subunit have been identified in many eucaryotic organisms (Lozeman *et al.*, 1990; Glover, 1998; Litchfield, 2003). Human cells contain two well characterized isoforms of the catalytic subunit, designated CK2α and CK2α'. A third one, the CK2α''-highly expressed in liver, has been identified recently (Shi *et al.*, 2001). Mammals contain a single regulatory CK2β subunit, but multiple forms of CK2β have been found in simple eukaryotes and plants (Glover, 1998; Pinna, 2002; Litchfield, 2003).

Protein kinase CK2 plays an important role in the cell, regulating many physiological processes, such as signal transduction, transcriptional control, proliferation, cell cycle control, and pathological ones like: viral diseases or cancer development (Litchfield, 2003).

CK2 is one of the most known pleiotropic protein kinase, able to phosphorylate over 307 proteins (Meggio and Pinna, 2003), whose phospho-acceptor sites are specified by multiple acidic residues downstream from the phosphorylable amino acid. The acidic side chain at position n+3 plays the most prominent role in susceptibility to CK2 phosphorylation (Pinna, 2002; Meggio and Pinna, 2003). One of the widest groups of the proteins phosphorylated by CK2 is formed by transcription factors. This group contains over 60 proteins. Between them are: c-Myb (Bergholts *et al.*, 2001), c-Myc (Channavajhala and Seldin, 2002), CREB (Saeki *et al.*, 1999), mdm2 (Gotz *et al.*, 1999), p53 (Mc Kendrick *et al.*, 1999), PU-1 (Lodie *et al.*, 1998), MAX, mUBF (Pinna *et al.*, 1995) and TFIIIA (Westmark *et al.*, 2002).

The Elf1 was recently identified as a regulatory protein involved in transcription elongation. It is functionally related to several other elongation factors and complexes, including Spt6, TFIIS, and the Pat complex. Elf1 contains a conserved zinc finger domain; implicated in the maintenance of proper chromatin structure in actively transcribed regions (Prether *et al.*, 2005). An *elf1* gene was also identified that function in controlling BMV translation - its deletion inhibits Brome mosaic virus (BMV) gene expression (Kushner *et al.*, 2003). As shown above protein kinase CK2 has been implicated in regulation of transcription. According to the amino acid sequence Elf1 harbours at least nine putative CK2 phosphorylation sites.

The present paper is a continuation of our studies on the structure and function of protein kinase CK2 from *Saccharomyces cerevisiae*. We report here cloning, overexpression, purification and phosphorylation of Elf1 protein. We show an interaction of transcription elongation factor Elf1 with subunits of CK2 and possible role of phosphorylation on Elf1 activity is discussed.

Materials and Methods

Strains, plasmids, enzymes and reagents. Yeast strain BMA64-1A (Euroscarf) was used as a source of the total yeast DNA for PCR amplification. *Escherichia coli* BL21 trxB (DE3) strain (Promega) was applied to expression of Elf1-Gst protein. The plasmid pGEX-4T-1 (Amersham Bioscences) was used for the construction of the expression system. Restriction enzymes, polimerase and ligase were purchased from Fermentas. All reagents were molecular biology grade and were purchased from Sigma. [γ-^{32}P]ATP (spec. act. 4500 TBq/mmol) was from MP Biomedicals (formely ICN Biomedicals Inc.).

Yeast elf1 cloning. The *elf1* coding sequence was amplified with PCR using yeast genomic DNA as a template applying standard protocol. Primers 5'-CGGGATCCGGTAAAAGAAAGAAGTCAAC-3' and 5'-ACGGTCGACATTATTCATCGTCACTATC-3' were designed for amplification of the *elf1* coding region flanked by *Bam*H1 and *Sal*1 restriction sites. The amplified product was purified, digested with *Bam*H1 and *Sal*1 and ligated into expression vector pGEX-4T-1. *E. coli* cells BL21 were transformed with ligation mixture. Plasmid pGEX-4T-1:: *elf1* isolated from transformed bacteria was sequenced in DNA Sequencing and Oligonucleotide Synthesis Laboratory IBB PAS in Warsaw to confirm integrity of sequence encoding yeast *elf1*.

Elf1 overexpression and purification. Overexpression of pGEX-4T-1::*elf1* was performed in one-liter bacterial culture in LB medium containing ampicilin (100 μg/ml) and kanamycin (25 μg/ml). Expression of the Elf1 protein was induced by IPTG addition (up to 0.1 mM) just after OD600$_{nm}$ value reached 0.5. After 5 h growth following induction, cells were harvested by centrifugation (5 min, 4°C, 10,000 × *g*) and stored at −70°C until use.

Bacterial cells (2 grams) were suspended in 15 milliliters of lysis buffer (50 mM Tris-HCl pH 7.5; 300 mM NaCl; 0.5 mM PMSF; 6 mM 2-mercaptoethanol) and sonicated in six 10 second cycles with 30 second breaks for cooling. Obtained suspension was then centrifuged (15 min, 4°C, 20,000 × *g*). Supernatant containing 12.5 mg of total protein was loaded twice onto Glutathione-Agarose beads (1 ml) following column rinsing with TBS buffer containing 1% Tween. Proteins elution was carried out with buffer containing 10 mM reduced glutathione in 50 mM Tris-HCl pH 9.4. Eluted proteins were collected in fractions (0.5 ml) and analyzed on 12,5% SDS/PAGE. Fractions containing purified Elf1-Gst protein were combined, dialyzed against buffer (50 mM Tris-HCl pH 7.5; 0.5

mM EDTA; 0.5 mM PMSF; 6 mM β-mercaptoethanol) and stored at −20°C for further experiments.

Protein kinases. Holoenzyme of protein kinase CK2 (hCK2) from *Saccharomyces cerevisiae* was prepared according to procedure described before (Szyszka *et al.*, 1986), followed by Heparin-Sepharose and α-Casein-Sepharose affinity chromatography to give preparation with near homogeneity.

Recombinant CK2α' was expressed from plasmid Yeplac181::*cka2*::*6his*::*5myc* (gift from Prof. H. Riezman, University of Geneva) in one-liter yeast culture in YPD medium.

CK2α' purification was conducted in two chromatography steps: DEAE-cellulose and HIS-SELECT cartridge (Sigma).

Three other recombinant CK2 subunits (CK2α, CK2β and CK2β') were expressed from pYES2/CT::*cka1*::*6his*::*v5*, pYES2/CT::*ckb1*::*6his*::*v5* and YEplac181::*ckb2*::*6his*::*5myc*, respectively. Purification of CK2 recombinants was carried out with one-step chromatography on HIS-SELECT cartridge.

Elf1 phosphorylation. Phosphorylation reactions were conducted at 30°C for 5-30 minutes in 50 µl samples containing 0.1-1.5 units of hCK2 or CK2α'; 0.25-2 µg Elf1 or Elf1-Gst; 20 µM [γ-^{32}P]ATP (specific radioactivity 500-1000 cpm/pmol); 15 mM Mg^{2+}; 20 mM Tris-HCl pH 7.5; 6 mM 2-mercaptoethanol. Reaction was terminated by additon 10 µl SDS/PAGE sample buffer (if phosphorylation was analyzed on SDS/PAGE) or 100 µl 10% TCA.

In order to calculate the level of phosphate incorporation into Elf1 protein, 0.5 µg Elf1-Gst was phosphorylated in the presence of 0.1-1.5 units of CK2α' at 30°C for 30 minutes. Reaction was terminated by addition 100 µl 10% TCA, samples were filtrated through GF/C membrane (Whatman) and radioactivity was counted in the scintillation counter.

For determination of K_m and V_{max} values, 0.25-2 µg of Elf1-Gst was phosphorylated in the presence of 1 unit of hCK2 or CK2α' at 30°C for 5 min. and counted as described above. Obtained data were analyzed with Lineweaver-Burke representation.

In order to confirm that Gst does not interfere with Elf1 protein phosphorylation by CK2, 10 µg of Elf1-Gst was digested with 1 unit of thrombin in 1 ml at room temperature for 2 h. 1 unit of CK2α' was added to 50 µl of reaction sample and phosphorylation reaction was performed at 30°C for 30 minutes. Phosphorylation was terminated by addition of 10 µl of SDS/PAGE sample buffer and proteins were resolved by electrophoresis following Coomassie Brilliant Blue staining. Dried gels were exposed to Kodak X-Omat film to detect ^{32}P-labelled bands.

One unit of protein kinase activity was defined as the amount of the enzyme required for incorporation of 1 pmol phosphate from [γ-^{32}P]ATP into a protein substrate per minute under conditions described above.

Mass spectrometric analyses. Two protein bands (about 4 µg of each) of phosphorylated and non-phosphorylated Elf1 protein (control sample) were excised from Coomassie blue-stained SDS/PAGE gel and digested *in situ* with porcine trypsin as described (Hellman, 2000). After digestion and extraction, a small aliquot of the peptide mixture was analysed by MALDI TOF. In case of peptides obtained from P-Elf1 the negative indications were obtained by peptide mass fingerprinting using an Bruker Ultraflex TOF/TOF mass spectrometer (Bruker Daltonics).

Phosphoamino acids analysis. 10 µg of ^{32}P-phosphorylated Elf1 protein was exposed to SDS/PAGE in 10% gel and then transferred onto PVDF membrane. Elf1 band stained previously with Ponceau Red solution was excised from membrane and placed in the hermetic glass vial containing 150 µl 6N HCl. Protein hydrolysis was conducted at 110°C for 1 h. After HCl evaporation sample was suspended in 5 µl of distilled water and loaded onto cellulose plate (20 cm × 20 cm, Merck). Two-dimensional electrophoresis was performed using formic-acetic buffer pH 1.9 in first dimension and pyridine buffer pH 3.5 in the second one, applying the high voltage current -1.5 and 1.6 kV respectively. Phosphoserine, phosphothreonine and phosphotyrosine were used as standards, and were visualized by ninhydrine staining (0.1% solution in aceton-acetic acid mixture). Positions of ^{32}P-labelled amino acids were detected by exposition of dried plates to Kodak X-Omat film to detect ^{32}P-spots.

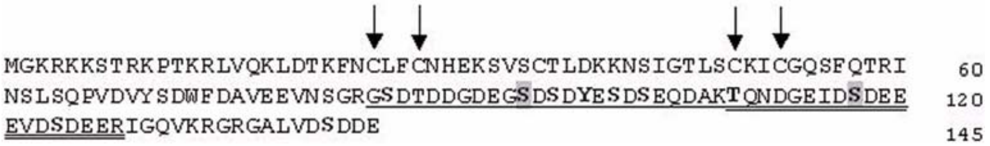
Co-immunoprecipitation of Elf1 and CK2 subunits. 15 µg of Elf1-Gst were mixed separately with 2 µg of each yeast CK2 subunits. Reaction was conducted at 4°C overnight in 200 µl TBS buffer. Next day 50 µl of Protein A-Agarose (50% slurry) was added to the samples followed by 1 hour incubation in 4°C with gentle mixing. Simultaneously four control reactions (samples without Elf1-Gst) were performed. Protein A-Agarose was centrifuged at 10,000 × *g* for 1 minute and pre-cleared samples were transferred to fresh tubes. 5 µg of monoclonal anti-Gst antibodies were added to each sample and immunoprecipitation was carried out at 4°C for 4 hours. 50 µg of 50% Protein A slurry was added to samples and after 2 hours of gentle mixing beads were centrifuged and washed 5 times with RIPA buffer containing: 50 mM Tris, pH 8; 150 mM NaCl; 0.1% SDS; 1% Triton X-100 and 0.5% Sodium deoxycholate. Protein A-Agarose was then resuspended in 100 µl of 2× SDS-PAGE sample buffer, boiled for 5 minutes and centrifuged. Supernatants were loaded onto 10% SDS/PAGE and electrophoresis was performed followed by Western Blot analysis.

Other procedures. Protein concentration was determined using Bradford reagent (Sigma) according to the manufacturer procedure with bovine serum albumin as a standard.

Results and Discussion

Protein kinase CK2 was discovered over 50 years ago (Pinna and Meggio, 1997) but its physiological roles was enigmatic for a long time and still remains incompletely understood (Glover, 1998; Pinna, 2002; Meggio and Pinna, 2003; Litchfield, 2003). There are over 300 proteins whose phosphorylation has been reported; among them 60 belong to group of transcription factors (Meggio and Pinna, 2003).

The starting point of presented work was searching of *Saccharomyces cerevisiae* database in order to find proteins which fulfil the criteria to be a protein substrate and possible protein interacting with CK2. Among several candidates we



MGKRKKSTRKPTKRLVQKLDTKFNCLFCNHEKSVSCTLDKKNSIGTLSCKICGQSFQTRI 60
NSLSQPVDVYSDWFDAVEEVNSGRGSDTDDGDEGSDSDYESDSEQDAKTQNDGEIDSDEE 120
EVDSDEERIGQVKRGRGALVDSDDE 145

Fig. 1. Yeast Elf1 amino acid sequence and positions of the serines phosphorylated by protein kinase CK2. The phosphorylated serine residues are in white with a gray background, the tryptic peptides containing the phosphoserines are underlined. Putative other CK2 phosphorylation sites are bold. Arrows indicate positions of four conserved cysteins of a zinc finger domain.

chose Elf1, which seems to play an important role in regulation of yeast transcription. The Elf1 protein amino acid sequence was analysed and compared with sequences of CK2 subunits. The N-terminal end of Elf1 protein contains sequence K^{3}RKKSTRK^{10} (Fig. 1) with high homology to the sequences K^{75}KKKIKRE^{82} and K^{85}MKKIYRE^{92} present in CK2α and CK2α' respectively. This α-helical lysine rich cluster is involved in substrate recognition of the CK2 catalytic subunits, inhibition by heparin and down-regulation exerted by the CK2β (Pinna and Meggio, 1997). The same basic cluster of CK2α seems to be responsible for nuclear targeting mediated by nuclear localization signal (NLS) (Nigg *et al.*, 1991). Second possible interaction cluster of Elf1 polypeptide is represented by amino acid sequence D^{112}GEIDSDEEE^{121} (Fig. 1). Very similar sequences can be found in CK2 regulatory subunits. They are D^{72}LEAMSDEEE^{81} and D^{84}DLDDSILEN^{93} and represent CK2β and CK2β' respectively. Mutagenesis studies on the β subunit revealed responsibility of this acidic cluster for the autoinhibitory effect and for the stimulation of CK2 by polycations (e.g. polylysine) (Meggio *et al.*, 1994). The acidic fragment of the Elf1 polypeptide is in addition incrusted by serine residue which comprises highly possible phosphorylation site (Ser^{117}) for protein kinase CK2 (Fig.1) (Meggio and Pinna, 2003).

Yeast Elf1 is a member of zinc family proteins and contains four conserved cysteins of a zinc finger domain namely Cys^{25}, Cys^{28}, Cys^{49} and Cys^{52} (marked by arrows, Fig.1). Zinc finger motif containing four cysteine residues is also present in regulatory β subunits where it mediates their dimerization (Boldyreff et al., 1996; Chantalat *et al.*, 1999).

Recombinant Elf1 protein was obtained as a hybrid protein with glutathione transferase (Gst) using *E. coli* expression plasmid pGEX-4T-1. Purification of protein was performed on Glutatione-Agarose beads column. As shown on Fig. 2A (lane 1) affinity purification step results in almost homogenous protein with molecular weight of about 44 kDa. Digestion of Elf1-Gst protein with thrombin gives pure band of 17 kDa Elf1 protein and 27 kDa protein band of Gst (Fig. 2A lane 2). Both forms of Elf1 proteins were subjected to phosphorylation catalysed by CK2 and it occurs that both are good substrates for this enzyme (Fig. 2B lanes 1 and 2).

According to the consensus sequence S/TXXD/E (Pinna, 2002; Meggio nad Pinna, 2003) the Elf1 polypeptide harbours several putative CK2 phosphorylation sites. Amino acid sequence of Elf1 polypeptide contains eight serine residues at positions 86, 95, 97, 101, 103, 117, 124 and 142, and one threonine residue at position 109 - possible targets for CK2 mediated phosphorylation. Recently CK2 has been reported to catalyze phosphorylation of the yeast nucleolar immunophilin Fpr3 at a tyrosyl residue (Tyr^{184}) fulfilling the consensus sequence of Ser/Thr substrates (Wilson *et al.*, 1997; Marin *et al.*, 1999). Similar CK2 phosphorylation site is present at position of Tyr^{99}. Five from these sites Ser: 86, 103, 124 and 142, and Thr 102 contain negative determinants (Meggio *et al.*, 1994) (Fig. 1). First, we determined a possible number of phosphorylated residues by calculation of phosphate incorporated per Elf1 molecule (Fig. 3). As shown the phosphate incorporation level was estimated as ~1.93 what suggests that phosphate can be incorporated maximally into 2 residues of Elf polypeptide. Moreover, the two-dimensional analysis of the modified amino acids indicated that CK2 phosphorylates only serine residues in Elf1 molecule (Fig. 4).

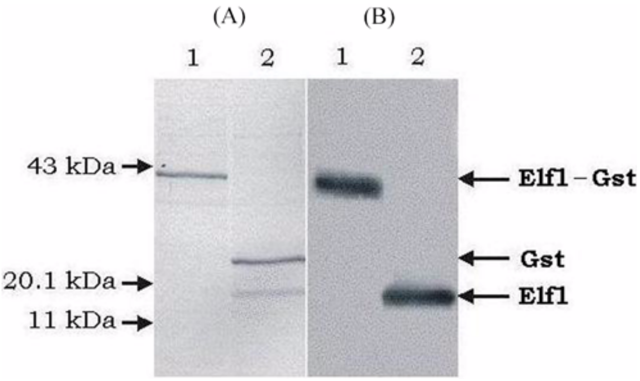


Fig. 2. Phosphorylation of Elf1 and Elf1-Gst by the recombinant CK2α'. 2 μg of pure recombinant Elf1-Gst (lanes 1 and 3) and Elf1-Gst after digestion with thrombin (lanes 2 and 4) were resolved in 12.5% SDS/PAGE followed by Coomassie blue staining (A) and autoradiography (B). On the left side arrows indicate positions of protein markers: ovoalbumin (43 kDa), soybean trypsin inhibitor (20.1 kDa) and acidic ribosomal protein P2 (11 kDa).

The major Elf1 phosphorylation sites were identified by MALDI-MS peptide analysis. Peptides obtained from phosphorylated *in vitro* and non-phosphorylated protein Elf1 were compared. For this purpose both forms of Elf1 polypeptide (non-phosphorylated and phosphorylated by CK2) were digested with trypsin and resulting peptide mixtures were analysed by MALDI-MS (Fig. 5A and B). As a

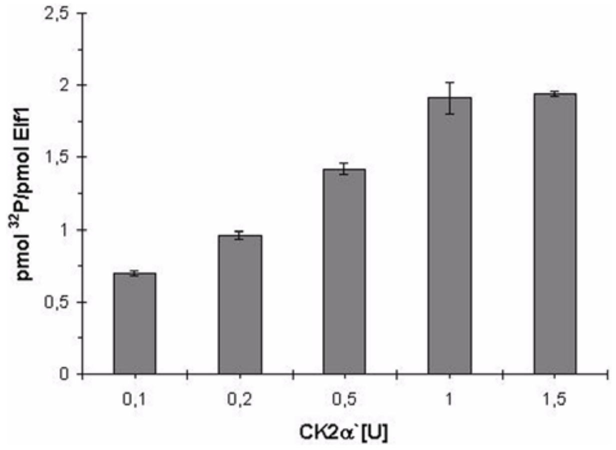


Fig. 3. Phosphate incorporation into Elf1 protein by CK2α'. 0.5 mg of pure recombinant Elf1-Gst protein were phosphorylated by recombinant CK2α' in the presence of phosphorylation buffer. Phosphorylated proteins were resolved in SDS/PAGE followed by Coomassie blue staining. The phosphate incorporation level was calculated by Cherenkov counting of ^{32}P-radioactivity incorporated to the Elf1-Gst protein. Data are means ± standard errors of the means of results from three experiments.

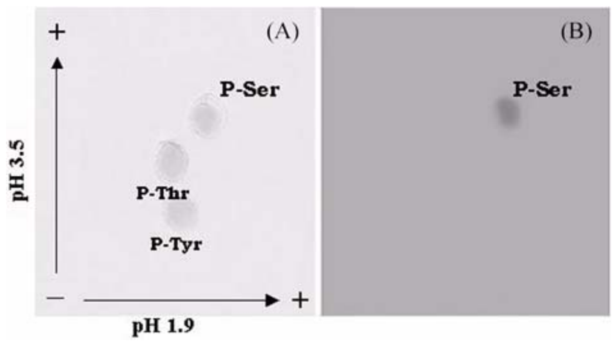


Fig. 4. Two-dimensional analysis of phosphorylated Elf1 amino acids. 30 μg of phosphorylated Elf1-Gst protein were separated on 10% SDS/PAGE, transferred onto PVDF membrane and hydrolysed in 6 N HCl. Amino acids obtained after hydrolysis were separated in two-dimensions. The standard phosphoamino acids were visualized by ninhydrine staining (A) and positions of ^{32}P-labeled amino acids were determined by autoradiography (B).

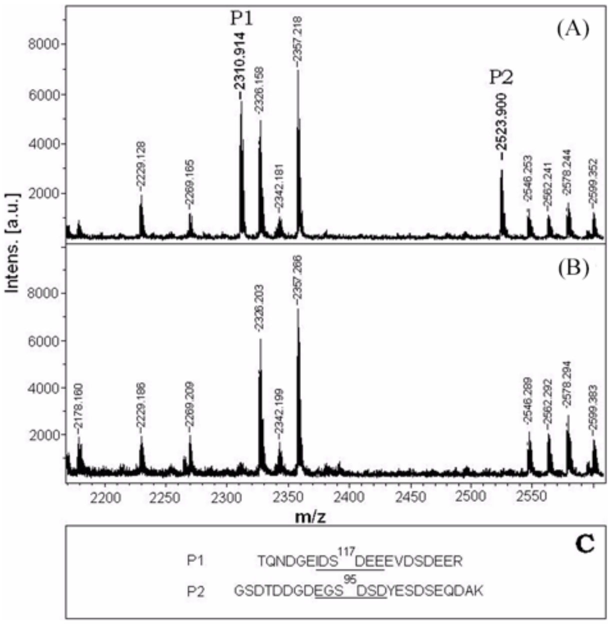


Fig. 5. Identification of phosphorylated peptides in Elf1. The protein band of non-phosphorylated (A) and phosphorylated (B) Elf1-Gst protein was excised from Coomassie blue-stained SDS/PAGE gel and digested *in situ* with porcine trypsin [33]. The peptide mixture was analysed by MALDI TOF. Amino acid sequences of phosphorylated peptides P1 and P2 are shown in part C.

result we obtained data indicating that phosphate substitution occurs in peptides with m/z signals 2310.914 (peptide P1) and 2523.900 (peptide P2). They correspond to the amino acid residues: T^{109}QNDGEIDSDEEEVDSDEER^{128} (P1) and G^{85}S DTDDGDEGSDSDYESDSEQDAK^{108} (P2) (Fig. 5C). Amino acid sequences of both peptides contain few CK2 consensus sites: two putative serine residues in P1 and five in P2, namely Ser^{117}, Ser^{124} in P1 and Ser^{86}, Ser^{95}, Ser^{97}, Ser^{101}and Ser^{103} in P2. Basing on results presented above and on the individual amino acid present within CK2 phosphoacceptor sites (Marin *et al.* 1999; Meggio *et al.*, 1999; Meggio and Pinna, 2003) and also on surface accessibility we conclude that the most probable phosphorylation sites modified by CK2 in Elf1 protein are Ser^{95} present in P2 and Ser^{117} from P1.

A large number of evidences indicate that a major mechanism for the regulation of specific protein kinases is represented by protein-protein interactions (Pawson and Nash, 2000). The regulation of wide range of different cellular processes requires involvement of specific protein interaction domains leading to direct association of polypeptides with each other and with small molecules, or nucleic acids.

Yeast protein kinase CK2 is a tetrameric enzyme composed of two catalytic (α and/or α') and two regulatory (β and β') subunits (Glover, 1998; Litchfield, 2003; Domanska *et al.*, 2005). As shown above in this paper transcription factor Elf1 is phosphorylated by CK2. To verify that the Elf1 protein and CK2 are functional in respect to their capacity to interact with each other, we demonstrate that each of the CK2 subunits, namely: CK2α, CK2α', CK2β and CK2β' can be detected in specific anti-Gst co-immunoprecypitates (Fig. 6). In case of catalytic α and α' subunits and the substrate protein Elf1, it is likely that the interaction with CK2 simply reflects enzyme-substrate interactions as it is observed in many other cases (Litchfield, 2003). Interaction between Elf1 and regulatory β/β' subunits of CK2 requires however another explanation. As we have mentioned before Elf1 contains on the N-terminal end very basic cluster represented be amino acids 3-10. This α-helical part of Elf1 is similar to the basic clusters of CK2α and CK2α' (amino acids 75-82 and 85-92 respectively). These region of CK2 catalytic subunits have been shown to interact

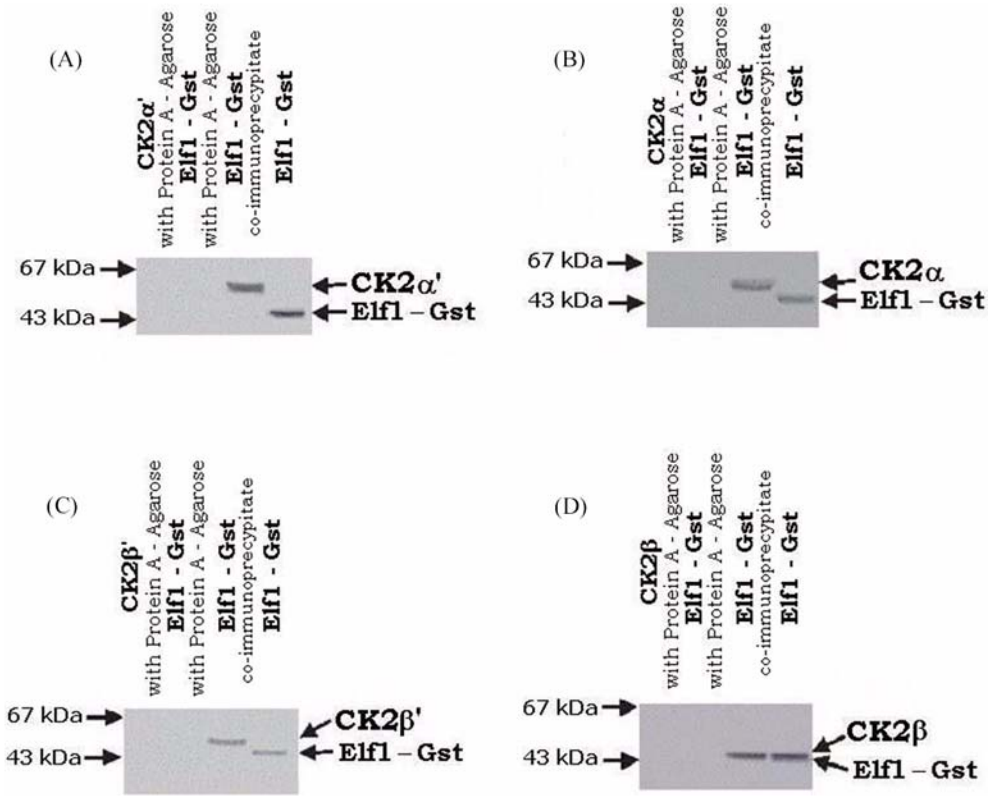


Fig. 6. Co-immunoprecipitation of Elf1-Gst and CK2 subunits. Samples obtained as result of co-immunoprecipitation (Materials and methods) were loaded onto 10% SDS/PAGE and electrophoresed followed by Western Blot analysis. Elf1-Gst was detected with anti-Gst monoclonal antibodies and CK2 subunits were detected with: anti-c-Myc monoclonal antibodies (CK2α' and CK2β') or with anti-V5 monoclonal antibodies (CK2α and CK2β). Arrows on the left side of each gel indicate positions of protein markers: bovine serum albumin (67 kDa), ovoalbumin (43 kDa). Lines 1 contain control samples (appropriate CK2 subunits with Protein A-Agarose); Lines 2 contain samples (Elf1-Gst with Protein A-Agarose); CK2 subunits detected with monoclonal antibodies after co-immunoprecipitation with Elf1 are shown on line 3 of each panel and line 4 contain Elf1-Gst protein detected with anti-Gst antibodies. To exclude interactions between CK2 subunits and Gst protein identical control experiment was done with samples containing Gst with Protein A-Agarose instead of Elf1-Gst with Protein A-Agarose but crossreaction between CK2 subunits and Gst protein was not observed (not shown).

with acidic clusters of regulatory β subunits in an autoinhibitory mode (Meggio *et al.*, 1994). Maybe similar mechanism should be employed to explain complex formation between Elf1 and β/β' subunits.

It has been reported that in many cases CK2 substrates are interacting partners of both the catalytic and regulatory subunits as it has been shown for eIF2b (Llorens *et al.*, 2003), FGF-1 (Skjerpen *et al.*, 2002) and Hsp90 (Suttitanamongkol *et al.*, 2002). Moreover there have been many observations showing that CK2 subunits mediate effects other phosphorylation acting as an adaptor/scaffold/targeting proteins (Pinna, 2002; Litchfield, 2003).

Since it was somewhat intriguing that Elf1 has ability to interact with catalytic as well as regulatory CK2 subunits, but is not only phosphorylated by the holoenzyme (hCK2), but also by the catalytic α' subunit alone we determine kinetic parameters for Elf1. The pure Elf1 protein was subjected to phosphorylation catalysed by both forms of yeast protein kinase CK2. The K_m value for the CK2α' subunit was 0.38 mM whereas for the holoenzyme it was 0.13 mM. The V_{max} values were not much different: 4.9 nmol/min/mg for the free CK2α' subunit and 4.1 nmol/min/mg for the holoenzyme. Elf1 has obviously higher affinity to the holoenzyme than CK2α' subunit, arguing for a positive influence of the β subunit.

Studies supporting results presented in this paper were presented very recently (Prather *et al.*, 2005). It was found that the purified Elf1 is associated with CK2 subunits α and β. In addition, it was observed that Elf1 is localized in regions of active transcription where affects chromatin structure in actively transcribed regions. Moreover, it was observed that Elf1 localization is partially dependent on proteins Spt4 and Spt6. The Spt4 protein forms a complex with Spt5 and mediates both activation and inhibition of transcription elongation, and plays a role in pre-mRNA processing (Rondon *et al.*, 2003). In our latest work we have presented that the Fip1 protein - a key regulator of mRNA polyadenylation process is also affected by CK2 phosphorylation what probably impairs its ability to function in the polyadenylation step (Zielinski *et al*., 2006). Results shown in this work together with experiments done lately by Prether and co-workers (Prether *et al*., 2005) indicate that Elf1 is a target for CK2 phosphorylation and probably this process may destabilize interaction with Pol II and proteins Spt4 and Spt6. As shown in this paper prominent role in recruitment and activity regulation of CK2 may have regulatory subunit β/β'. Further work will be needed to confirm this hypothesis and will require an accurate analysis of relations between all proteins of this complex.

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