Protein kinase CK2 potentiates translation efficiency by phosphorylating eIF3j at Ser127

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ABSTRACT

In eukaryotic protein synthesis the translation initiation factor 3 (eIF3) is a key player in the recruitment and assembly of the translation initiation machinery. Mammalian eIF3 consists of 13 subunits, including the loosely associated eIF3j subunit that plays a stabilizing role in the eIF3 complex formation and interaction with the 40S ribosomal subunit. By means of both co-immunoprecipitation and mass spectrometry analyses we demonstrate that the protein kinase CK2 interacts with and phosphorylates eIF3j at Ser127. Inhibition of CK2 activity by CX-4945 or down-regulation of the expression of CK2 catalytic subunit by siRNA cause the dissociation of eIF3j from the eIF3 complex as judged from glycerol gradient sedimentation. This finding proves that CK2-phosphorylation of eIF3j is a prerequisite for its association with the eIF3 complex. Expression of Ser127Ala–eIF3j mutant impairs both the interaction of mutated j-subunit with the other eIF3 subunits and the overall protein synthesis. Taken together our data demonstrate that CK2-phosphorylation of eIF3j at Ser127 promotes the assembly of the eIF3 complex, a crucial step in the activation of the translation initiation machinery.

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1. Introduction

In eukaryotic protein synthesis, initiation of translation is a complex sequence of reactions requiring the interaction of the ribosome with a number of eukaryotic translation initiation factors (eIFs). A key player in the recruitment and assembly of the translation initiation machinery is the multiprotein complex eIF3, which stimulates many steps of the pathway. These include assembly of the eIF2-GTP/met-tRNAi complex and of other eIFs to the 40S ribosomal subunit to form the 43S preinitiation complex, recruitment of mRNA to the 43S complex, prevention of the 40S ribosome from joining the 60S prematurely, and the scanning of mRNA for AUG recognition [reviewed in 1,2]. In mammals, eIF3 contains 13 different subunits, which are named IF3α to IF3m in order of decreasing molecular weight, and possesses an anaphoromorphic five-lobed structure [3] organized around a functional core complex [4,5]. Structural analysis suggests that eIF3 performs a scaffolding function by binding to the 40S subunit on its solvent-exposed surface rather than on its interface with the 60S subunit, where the decoding sites are located [3]. This location of eIF3 seems ideally suited for its other proposed regulatory functions, including its acting as a receptor for protein kinases that control protein synthesis [6,7]. eIF3j is a nonstoichiometric and highly conserved subunit, that is loosely associated with the eIF3 complex [8,9]. It makes multiple independent interactions with the eIF3 core [5] and its binding to the eIF3b N-terminal RNA recognition motif plays a stabilizing role in forming the eIF3 complex [10]. eIF3j is required for high-affinity binding of eIF3 to the 40S ribosomal subunit, it associates with the decoding center of the 40S subunit and governs the binding of initiation factors and mRNA to form a scanning-competent initiation complex [8,9,11–13]. It has been also proposed that eIF3j promotes mRNA dissociation during the ribosomal recycling step [14].

Protein kinase CK2 is a ubiquitous, highly conserved and pleiotropic Ser/Thr kinase, endowed with constitutive activity, independent of any known second messenger or phosphorylation events. The kinase is usually present as a tetrameric holoenzyme composed of two catalytic subunits (α and/or α′) and two non-catalytic (β-subunits. CK2 phosphorylates a huge number of protein substrates, implicated in fundamental cell processes. Among the CK2 substrates there are also transcription factors, modulators of DNA and RNA structure, and proteins involved in RNA and protein biosynthesis, which highlight the
importance of CK2 in controlling gene expression [15,16]. CK2 is abnormally elevated in a wide variety of tumors, where it plays a global role as an anti-apoptotic and pro-survival agent operating as a cancer driver by creating a cellular environment favorable to neoplasia [17–19].

In this study we show that the protein kinase CK2 interacts with and phosphorylates the j subunit of the elf3 complex. CK2 catalyzed phosphorylation of elf3j triggers the association of this subunit with elf3 complex promoting an efficient translation initiation.

2. Materials and methods

2.1. Materials and antibodies

[γ-32P]ATP was purchased from Hartmann Analytic GmbH (Braunschweig Germany). Protease inhibitor cocktail was from Calbiochem (Darmstadt, Germany), whereas phosphatase inhibitor cocktails 2 and 3 and the kit for molecular weight markers were from Sigma-Aldrich (Darmstadt, Germany), while phosphatase inhibitor cocktails 2 and 3, Schweig Germany). Protease inhibitor cocktail was from Calbiochem (Darmstadt, Germany).

2.2. Cell lysis and western blot analysis

Cells were lysed by suspension (1 h at 4 °C) in the lysis buffer containing 20 mM Tris–HCl (pH 7.5), 1% Triton X-100, 10% glycerol, 1 mM EDTA, 150 mM NaCl, and protease and phosphatase inhibitor cocktails. After centrifugation (16,000 × g for 15 min), protein concentration was determined in the supernatants by Bradford method. Proteins were subjected to 11% SDS-PAGE, blotted on Immobilon-P membranes (Sigma-Aldrich), processed in western blot with the indicated antibodies and developed using an enhanced chemiluminescent detection system (ECL). Immunostained bands were quantified by means of a Kodak-Image-Station 4000MM-PRO and analysis with Carestream Molecular Imaging software (New Haven, CT).

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2.4. Immunoprecipitation experiments

Proteins from cell lysates (400 μg in 250 ml) were immunoprecipitated overnight with the specific antibody, followed by addition of protein A-Sepharose for 30 min. The immunocomplexes, washed three times with 0.9 ml of 50 mM Tris–HCl, pH 7.5, were analyzed by western-blot or tested for CK2 activity by in vitro phosphorylation assay.

2.5. Phosphorylation assay of immunoprecipitates

Anti-CK2α, anti-elf3j and anti-Myc immunoprecipitates were phosphorylated in 25 μl of a phosphorylation medium containing 50 mM Tris–HCl (pH 7.5), 10 mM MgCl2, 100 mM NaCl and 20 μM [γ-32P]ATP (about 1000 c.p.m./pmol). Samples were subjected to SDS-PAGE and blotted. Radioactive proteins were evidenced by a Cyclone Storage Phospho-Screen (PerkinElmer, Waltham, MA).

2.6. Identification of the elf3j site phosphorylated by CK2

HEK293 cells, treated with vehicle or CX-4945 were immunoprecipitated with elf3j antibody. Immunocomplexes were loaded on SDS-PAGE and the bands corresponding to the MW of about 35–38 kDa were excised from the gel and digested by trypsin (for details see Supplementary Materials and Methods). The resulting peptides were subjected to a phosphopeptide enrichment step. Samples were dried under vacuum and dissolved in 30 μl of a loading buffer that was constituted of 80% acetonitrile (ACN) and 6% trifluoroacetic acid (TFA, riedel-de Haen). Samples were slowly loaded onto homemade TiO2 micro-columns (prepared as described in [23]) pre-conditioned twice with 20 μl of ACN and twice with 20 μl of loading buffer. Micro-columns were then washed twice with 20 μl of loading buffer and twice with 0.1% TFA (20 μl each time) to increase the pH. Phosphopeptides bound to the stationary phase were finally eluted with 20 μl of freshly prepared NH4OH (5%, pH ≈ 11), and released from the C18 frit with a further elution step using 50% ACN, 0.1% formic acid (FA). 2 μl of pure FA was finally added to acidify the samples. Phosphopeptides were dried under vacuum and dissolved in 0.1% FA for mass spectrometry (MS) analysis that was conducted as described in [24].

Data were analyzed with Proteome Discoverer software (version 1.4, Thermo Fisher Scientific) coupled to a Mascot search engine (version 2.2.4, Matrix Science) against the human section of the Uniprot database (release 20140416, 88708 entries). (For further details see the Supplementary Materials and Methods.) The MS/MS spectra of identified phosphopeptides were manually inspected for confirmation.

2.7. Glycerol gradient sedimentation

Cells were lysed by suspension (1 h at 4 °C) in the lysis buffer containing 20 mM Tris–HCl (pH 7.5), 1% Triton X-100, 10% glycerol, 1 mM EDTA, 150 mM NaCl, and protease and phosphatase inhibitor cocktails. After centrifugation (16,000 × g for 15 min), protein concentration was determined in the supernatants by Bradford method. Proteins were subjected to 11% SDS-PAGE, blotted on Immobilon-P membranes (Sigma-Aldrich), processed in western blot with the indicated antibodies and developed using an enhanced chemiluminescent detection system (ECL). Immunostained bands were quantified by means of a Kodak-Image-Station 4000MM-PRO and analysis with Carestream Molecular Imaging software (New Haven, CT).

2.8. RNA interference

HEK293 cells (5 × 105) were transfected for 72 h with 30 nM CK2α and CK2β specific siGENOME SMARTpool siRNAs (Dharmacon, Lafayette, CO, USA) or non-specific siRNA siCONTROL rescifree#1 (Dharmacon). Transfection was performed using the transfecting reagent INTERFERin (Polyplus-transfection SA, Illkirch, France) according to the manufacturer’s recommendations.

2.9. CK2 kinase activity assay

Proteins from cell lysates were incubated for 10 min at 30 °C in 25 μl of a phosphorylation medium containing 50 mM Tris–HCl (pH 7.5), 100 mM NaCl, 12 mM MgCl2, 400 μM synthetic peptide-substrate RRADSDDDDDD and 20 μM [γ-32P]ATP (1000 c.p.m./pmol). Assays
were stopped by absorption onto phosphocellulose filters. Filters were washed four times in 75 mM phosphoric acid [21] and analyzed by a Scintillation Counter (PerkinElmer).

2.10. Plasmids and cell transfection

pCMV-Myc vector, pCMV-Myc-wild type eIF3j and pCMV-Myc-S127A-eIF3j were purchased from GenScript (Piscataway, NJ, USA). HEK293 cells (1 × 10^6) were transfected for 96 h with 4.5 μg of the indicated constructs, using TransIT-LT1 (Mirus Bio LLC, Madison, WI, USA) according to the protocol recommended by the manufacturer.

2.11. 35S-methionine/cysteine metabolic labeling

HEK293 cells were transfected as described in Section 2.10 and then cycloheximide (100 μg/ml) was added to a cell medium for 1 h. Then the medium was replaced by Met/Cys-free DMEM (Sigma-Aldrich) supplemented with 2 mM L-glutamine and 100 μg/ml cycloheximide. After 1 h, the medium was replaced by Met/Cys-free DMEM supplemented with 2 mM L-glutamine and 10 μCi/ml [35S]-L-Met/Cys Protein Labeling Mix (Hartmann Analytic GmbH). After 1 h, radiolabeled medium was removed and cells were washed and lysed as described in Section 2.3. Similar amounts of radioactive lysates were subjected to SDS-PAGE, blotted and the total labeling was measured using the Cyclone Plus Storage Phosphor System.

2.12. Statistical analysis

Data are presented as means ± SD and mean differences were analyzed using t-test. A p < 0.05 was considered as statistically significant.

3. Results

3.1. j-Subunit of eIF3 is a new interactor and substrate of CK2

HEK293, Hela and LAMA84 cells were analyzed in experiments aimed at identifying new CK2 substrates. Cellular lysates were immunoprecipitated with an antiseraum raised against the human CK2α C-terminus. Immunocomplexes were then phosphorylated in a medium containing [γ-33P]ATP. Fig. 1A shows that different co-immunoprecipitated proteins are 33P-phosphorylated including the expected CK2 regulatory β-subunit (lanes 1,4,7). The strong inhibition of the 33P-phosphorylation induced by in vitro addition of the CK2 selective and potent inhibitor CX-4945 [25] demonstrates that most proteins are specifically phosphorylated by the constitutively active CK2 (lanes 2,5,8). Consistently, addition of staurosporine at a concentration ineffectual toward CK2 but able to inhibit most protein kinases [26] does not affect the protein phosphorylation extent (Fig. 1A, lanes 3,6,9). The radioactive protein displaying a MW of about 35–38 kDa, which is highly phosphorylated and present in all the analyzed immunoprecipitates, was excised from the gel, digested by trypsin and analyzed by mass spectrometry. The analysis showed that, among the identified proteins, the j-subunit of the eukaryotic translation initiation factor 3 (eIF3j) (MW = 29,159) displayed the highest mascot score (see Supplementary Materials and Methods and Supplementary Table 1). The occurrence of an interaction between CK2 and eIF3j was further supported by the presence of CK2α and CK2β subunits in eIF3j-immunoprecipitates obtained from HEK293 cell lysates (Fig. 1B). The recognition of the co-immunoprecipitated CK2β subunits was confirmed by running recombiant CK2 holoenzyme (α2β2) in parallel with the eIF3j-immunoprecipitates. The identification of eIF3j as a new substrate of CK2 was strengthened by in vitro phosphorylation experiments performed with eIF3j-immunoprecipitates showing that j-subunit is phosphorylated by its interactor CK2. The kinase co-immunoprecipitated with eIF3j (upper panel of Fig. 1C) specifically phosphorylates the subunit as demonstrated by the strong reduction of the eIF3j 33P-phosphorylation caused by in vitro addition of the CK2 inhibitor CX-4945 (lower panel of Fig. 1C).

The finding that all the other eIF3 subunits were identified by mass spectrometry analysis among the proteins co-immunoprecipitated with CK2α (see Supplementary Materials and Methods and Supplementary
Table 2) supports the view that CK2 interactome contains the whole elf3 complex.

3.2. CK2 phosphorylates elf3j at Ser127

To check if elf3j is phosphorylated by CK2 inside the cell, HEK293 cells were treated with vehicle or CX-4945 for 5 h and then lysed. Lysate proteins were immunoprecipitated with anti-elf3j antibody and the immunocomplexes were subjected to SDS-PAGE. Protein bands corresponding to j-subunit were excised and digested with trypsin. Resulting phosphopeptides were enriched using TiO2 microcolumns and analyzed by tandem mass spectrometry, as described in Section 2.6. Fig. 2 shows the extracted ion chromatograms for several identified peptides belonging to elf3j. The elf3j phosphopeptide LQEpSDLELAK was identified in the control sample (panel A) but not in the sample immunoprecipitated from cells treated with the CK2 inhibitor CX-4945 (panel B). The ion at m/z = 677.8087 is indeed detectable only in the control experiment, and it is absent in the sample derived from cells treated with CX-4945, while all other ions are present in both samples with similar intensities. The MS/MS spectrum of the ion at m/z = 677.8087 (Fig. 2C) confirms the identification of the elf3j tryptic phosphopeptide LQEpSDLELAK containing the residue Ser127, which is located in a sequence matching the CK2 consensus, which is S/T-X-X-Acidic residue [16,27].

3.3. Inhibition of CK2 increases its binding to elf3j

Next we assessed whether the inhibition of CK2 activity might affect the interaction occurring between the kinase and elf3j. To this purpose, HEK293 cells were untreated or treated with CX-4945 or quinalizarin (Q1), two structurally unrelated CK2 inhibitors. Western blot analysis of cellular lysates demonstrates that CX-4945 and Q1 strongly reduce the phosphorylation extent of Akt1 Ser129, a specific target of CK2 [28] (about 80% and 65%, respectively), while they do not change the protein-level of CK2α, CK2β and elf3j (Fig. 3A). Cellular lysates were then immunoprecipitated with anti-CK2α antibody. Interestingly, while cell treatment with CX-4945 or quinalizarin does not affect the interaction of the kinase with the elf3 subunits elf3b and elf3d, it causes an about 3-fold increase of elf3j co-immunoprecipitation (Fig. 3B). This finding is confirmed by elf3j-immunoprecipitates showing a great enhancement of CK2α and CK2β co-immunoprecipitation in...
inhibitor-treated as compared to untreated cells, while the amounts of co-immunoprecipitated eIF3b and eIF3d are unchanged (Fig. 3C).

To assess whether eIF3j is bound directly to CK2α and/or CK2β, preliminary experiments were performed by down-regulating separately the amount of these two CK2 subunits in HEK293 cells by RNA-interference experiments. Cellular protein-level of CK2α and CK2β was reduced of about 48% and 43%, respectively (Fig. S1A of Supplementary Material). Co-immunoprecipitation of the individual CK2 subunits was then quantified in eIF3j-immunoprecipitates obtained from the lysates of the differently treated cells. Supplementary Material Fig. S1B shows that, in comparison with control cells, the eIF3j-immunoprecipitates obtained from CK2α-downregulated cells contain the expected decreased level of CK2α and a similar amount of CK2β.

On the contrary, CK2β-downregulation causes a substantial reduction of eIF3j interaction with both CK2 subunits, suggesting that the regulatory β-subunit might play a role as a linker between eIF3j and the CK2 catalytic α-subunit.

### 3.4. CK2-phosphorylation of j-subunit promotes its interaction with eIF3 complex

To get further insight into the interaction occurring between CK2 and eIF3j, HEK293 cells, treated with vehicle or CX-4945, were first lysed and subjected to sucrose gradient centrifugation in the presence of MgCl₂ as described elsewhere [28]. Under these conditions, most eIF3j is detectable in the low-density fractions, dissociated from the eIF3 complex (unpublished data and Ref. [2]). To recover the j-subunit included in the eIF3 complex, cells were therefore lysed and loaded on glycerol gradients as detailed in Section 2.7. In control cells, a part of

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**Fig. 3.** Effect of CK2 inhibition on its interaction with eIF3j. (A,B,C, left panels) HEK293 cells were treated with vehicle (DMSO), 2.5 μM CX-4945 or 5 μM quinalizarin (Q1) for 5 h and lysed. (A) Cellular lysates were analyzed by western blot with the indicated antibodies. (B,C) Cellular lysates were immunoprecipitated with (B) pre-immune serum (Ctrl) or anti-CK2α antibody, and (C) with anti-Fyn antibody of the same class or anti-eIF3j antibody. Immunocomplexes were then analyzed by western blot with the indicated antibodies. (A,B,C, right panels) Panels report the densitometric values ± SD relative to the phosphorylation extent of Akt1 phospho-Ser129 (A) and to the amount of the co-immunoprecipitated eIF3j (B) or CK2α (C). Values are expressed as percentage relative to the densitometric value of the bands observed in cells treated with vehicle. *p < 0.05. Figures are representative of at least four independent experiments.

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**Fig. 4.** Effect of CK2 inhibition by CX-4945 on eIF3j interaction with the eIF3 complex in HEK293 cells. (A,B) HEK293 cells were treated with vehicle ( Ctrl) (A) or 3 μM CX-4945 for 5 h (B). Cells were lysed and lysate proteins were separated on glycerol gradients as detailed in Section 2.7. The following molecular weight standards were run on separate tubes: β-amylase (200 kDa), apoferritin (443 kDa) and thyroglobulin (669 kDa). Gradients were fractionated into 19 fractions. 40 μl of the indicated fractions was loaded on the same gel, subjected to SDS-PAGE and analyzed by western blot. (C) Fractions 2 and 3 of the gradients relative to untreated (Ctrl) or CX-4945-treated cells were pooled and immunoprecipitated with anti-rpS6 antibody. Immunocomplexes were analyzed by western blot. (D) Fractions 7 and 8, or 12 and 13 of the gradients relative to untreated (Ctrl) or CX-4945-treated cells were pooled and immunoprecipitated with anti-eIF3j antibody. Immunocomplexes were analyzed by western blot. Figure is representative of five independent experiments.
the eIF3 complex appears in the high-density fractions of the gradient, bound to the 40S ribosomal subunit as indicated by the co-sedimentation and the co-immunoprecipitation of the S6 ribosomal protein (rpS6) with the eIF3 subunits b, c, d and j (fractions 1–4 of Fig. 4A, and Ctrl of Fig. 4C, respectively). Most of the complex is detectable in the gradient fractions containing protein complex(es) displaying a molecular weight higher than 400 kDa (Fig. 4A, fractions 6–9). The distribution of CK2α and CK2β subunits shows that only a small amount of the protein kinase co-migrates with the eIF3 complex in fractions 7–9 (Fig. 4A), while the majority of CK2 participates in complex(es) displaying a lower molecular weight (fractions 10–13). Notably, cell treatment with the CK2 inhibitor CX-4945 induces the dissociation of a large amount of j-subunit from the eIF3 complex both bound or unbound to the 40S subunits (Fig. 4B) and its shift toward the low-density region of the gradient, where CK2α and CK2β are also detectable (fractions 11–14). The finding that cell treatment with CX-4945 does not affect the protein-level of CK2 (Fig. 3A) suggests that eIF3j migration is induced only by inhibition of the kinase activity.

The effect of cell treatment with CX-4945 was further elucidated by immunoprecipitating specific fractions of the gradients. Immunoprecipitation of rpS6 from the high-density region of the gradient (fractions 2 and 3) (Fig. 4C) demonstrates that CK2-inhibition triggers the detachment of eIF3j from the protein-complex containing the other eIF3 subunits and the 40S ribosomal subunit. Immunoprecipitation of eIF3j (Fig. 4D) supports the previous finding that cell treatment with CX-4945 induces an increased interaction of eIF3j with inactive CK2 (Fig. 3). This is evident for both the populations of eIF3j, the one bound to the eIF3 complex (Fig. 4D, fractions 7,8) and that migrated to the low-density region of the gradient (fractions 12,13). The outcome that CK2 inactivation correlates with the j-subunit dissociation from the eIF3 complex supports the view that the CK2-phosphorylation of eIF3j is a prerequisite for the binding of this subunit to eIF3 complex.

Considering the importance of cell translational activity in cancer, the interplay occurring between CK2 and eIF3j was further analyzed also in LAMA84, a chronic myeloid leukemia cell line presenting an aberrant protein synthesis [29]. Fig. 1A demonstrates that eIF3j is an interactor and an in vitro substrate of CK2α. Immunoprecipitation of eIF3b and eIF3c in the two differently treated LAMA84 cells were loaded on two different gels, and analyzed by western blot under the same conditions. The effect of cell treatment with CX-4945 was further elucidated by immunoprecipitating specific fractions of the gradients. Immunoprecipitation of rpS6 from the high-density region of the gradient (fractions 2 and 3) (Fig. 4C) demonstrates that CK2-inhibition triggers the detachment of eIF3j from the protein-complex containing the other eIF3 subunits and the 40S ribosomal subunit. Immunoprecipitation of eIF3j (Fig. 4D) supports the previous finding that cell treatment with CX-4945 induces an increased interaction of eIF3j with inactive CK2 (Fig. 3). This is evident for both the populations of eIF3j, the one bound to the eIF3 complex (Fig. 4D, fractions 7,8) and that migrated to the low-density region of the gradient (fractions 12,13). The outcome that CK2 inactivation correlates with the j-subunit dissociation from the eIF3 complex supports the view that the CK2-phosphorylation of eIF3j is a prerequisite for the binding of this subunit to eIF3 complex.

3.5. S127A mutation of eIF3j affects the subunit interaction with eIF3 complex and impairs the protein synthesis

To further analyze the role played by eIF3j Ser127, HEK293 cells were transfected with MYC-tagged cDNA of either wild type eIF3j or the mutated counterpart containing Ser127 replaced by Ala. Fig. 7A shows that transfected cells overexpress the same amount of Myc-tagged proteins without affecting the expression of endogenous eIF3 subunits b and d. To reinforce the concept that Ser127 is indeed a CK2 target, wild type and mutant eIF3j were immunoprecipitated with anti-Myc antibody and the immunocomplexes were added to a phosphorylation medium containing recombinant CK2. The finding that S127A-eIF3j mutant is only slightly 32P-phosphorylated by the kinase in comparison with the wild type subunit proves that Ser127 is the main site of CK2-phosphorylation (Fig. 7B).

We then analyzed the interaction occurring between overexpressed j-subunit and endogenous eIF3 subunits by immunoprecipitation with wild type or mutant Myc-eIF3j with anti-Myc antibody (Fig. 7C). S127A mutation induces a substantial decrease of eIF3b, eIF3c and eIF3d binding to mutated eIF3j in comparison with the interaction detectable with wild type eIF3j, confirming the role played by Ser127 in eIF3 binding to the eIF3 complex. Consistently, the parallel eIF3b-immunoprecipitation (Fig. 7D) shows a similar co-immunoprecipitation of eIF3c and eIF3d in the two differently transfected cells, while the binding of the Myc-tagged proteins is different, being the amount of coimmunoprecipitated S127A-eIF3j lower than that of wild type eIF3j.
The deletion of the eIF3j ortholog HCR1 in Saccharomyces cerevisiae has been demonstrated to cause a significant decrease of the yeast growth rate [10,30], while the knocking down of eIF3j by siRNA in mammalian cells was found less effective on translational rate and cellular growth [2]. We wanted to assess whether the efficiency of the protein synthesis was affected by eIF3j S127A mutation. HEK293 cells, transfected with empty vector or overexpressing a similar amount of wild type or mutated Myc-eIF3j (Fig. 7E, upper panel), were pulsed in the presence of a radiolabeled methionine and cysteine mix before quantification of total protein labeling. Fig. 7E (bar graph) shows that the overall protein synthesis of control cells (Ctrl) is not affected by the overexpression of wild type Myc-eIF3j, demonstrating that eIF3j Ser127 plays a regulatory role in the translational control of protein synthesis.

4. Discussion

In this study we demonstrate by means of both immunoprecipitation experiments and mass spectrometry analyses that eIF3 complex is associated with CK2 in human cells and that eIF3j subunit is a new substrate of this protein kinase (Fig. 1). Although two eIF3 polypeptides isolated from rabbit reticulocytes and displaying the molecular weights of 70,000 and 120,000, have been described as the first CK2 substrates in very early studies [31,32], no other evidence has been subsequently provided that human eIF3 is regulated by CK2. It has been reported that eIF3c is a substrate of CK2 in plants [33] and that the yeast homologues of eIF3b and eIF3c are phosphorylated by CK2 [34]. Here we demonstrate that CK2 phosphorylates the eIF3 j-subunit at Ser127, a residue located in the sequence LQEEpDLAK matching the CK2 phosphorylation consensus sequence. This is specified by a carboxylic or pre-phosphorylated amino acid at position n + 3 with respect to the target residue and optimized by additional acidic amino acids in the proximity of the phosphorylatable residue [16,27]. Phosphoproteomic studies have already demonstrated that eIF3 Ser127 is phosphorylated in cells [35–38]. Our results show that this residue is specifically phosphorylated by CK2 since the eIF3 Ser127 phosphorylation does not occur in cells treated with the CK2-inhibitor CX-4945.
(Fig. 2) and the phosphorylation of elf3j is almost abrogated when Ser127 is replaced by Ala (Fig. 7B). Phospho-proteomic analyses have also shown that, like elf3j, other mammalian elf3 subunits contain phospho-residues located in sequences potentially susceptible to phosphorylation by CK2 [35,37], suggesting a global role of CK2 in the regulation of elf3, a central player in the mechanism of translational control, whose alterations may lead to either cell malignancy or cell death [39,40]. With regard to this, the notion that CK2 is abnormally elevated in a wide variety of tumors, where it is exploited as an anti-apoptotic and pro-survival agent, suggests that the oncogenic potential of this protein kinase might also rely on its ability to functionally affect elf3, whose contribution to oncogenesis and maintenance of the malignant phenotype has been amply demonstrated [41–43].

Cell treatment with CK2 inhibitors demonstrates that, once the kinase activity is inhibited, CK2 greatly enhances its binding to j-subunit (Figs. 3 and 5). Notably, this binding increase is partially due to a sub-population of j-subunit molecules that dissociate from elf3 complex following CK2 inhibition and co-migrate with inactive CK2 as demonstrated by differential sedimentation in glycerol gradients obtained with HEK293 and chronic myeloid leukemic LAMA84 cells (Figs. 4 and 5). The finding that treatment of LAMA84 cells with CX-4945 induces the detachment of elf3j from the preinitiation complex suggests that this event is involved in the decreased viability caused in these leukemic cells by CX-4945—inhibition of CK2 activity [44]. Consistently, the down-regulation of cellular CK2 by knocking-down the CK2α catalytic subunit in HEK293 cells by siRNA induces a similar dissociation of j-subunit from elf3 complex (Fig. 6C and D). These results are in agreement with studies showing that elf3j is a loosely associated subunit, easily detachable from the rest of the elf3 complex during purification and detectable in cells also as unbound protein [9,12]. The finding that CK2 inhibition promotes both the dissociation of j-subunit from elf3 and its increased binding to the kinase suggests that: i) non-phosphorylated elf3j binds to CK2 more than its phosphorylated counterpart; ii) elf3j, once phosphorylated by CK2, dissociates from the kinase; iii) elf3j dissociation from the elf3 complex is likely caused by the reduced CK2 activity and not by the decreased protein-level of the kinase (Fig. 6), as demonstrated in cells treated with CX-4945 (Figs. 4 and 5), that contain an unchanged amount of CK2 (Fig. 3A). This hypothesis is also supported by the reduced interaction of j-subunit with the elf3 complex occurring when active CK2 is unable to phosphorylate elf3j due to Ser127Ala mutation (Fig. 7); iv) CK2-phosphorylation of elf3j triggers the interaction of this subunit with the other complex subunits, an event that has been described to play a stabilizing role in forming the elf3 complex [9,10,13] as well as in the elf3 binding with the decoding center of the 40S ribosomal subunits [8,9,11–13]. It has been also recently shown that elf3j and elf3 complex bind cooperatively to the 40S ribosomal subunit and that the whole elf3 complex is critical for the high affinity binding of elf3j on the 43S preinitiation complex [13].

In mammals, the recruitment and positioning of Met-tRNAi on the small 40S ribosomal subunit to form the 43S preinitiation complex are mediated and stabilized by the eukariotic initiation factors elf1, elf1A, elf2, elf3 and elf5 [45,46]. It has been demonstrated that CK2 interacts with and phosphorylates human elf2b and elf5 affecting the translation initiation complex formation and the cellular growth rate [47,48]. Our results showing that also elf3j is phosphorylated by CK2 reveal that most of the initiation factors acting in mammalian preinitiation complex are regulated by CK2.

The key role played by elf3j Ser127 in elf3 complex assembly and translation initiation machinery is further supported by the data obtained upon transfection of HEK293 cells with a plasmid encoding S127A-elf3j mutant. We found that the amount of endogenous elf3 subunits interacting with S127A-elf3j is much lower than that detected in cells overexpressing wild type elf3j (Fig. 7C and D). Our results also show that protein synthesis is markedly reduced in cells containing S127A-elf3j mutant in comparison with cells overexpressing wild type elf3j (Fig. 7E), consistent with the notion that the main mechanism regulating the overall rate of protein synthesis involves the phosphorylation of the initiation factors [39].

Collectively taken, our data demonstrate that CK2 phosphorylation of elf3j at Ser127 regulates the j-subunit association with elf3 complex and underscores the crucial role of CK2 in the initiation of protein synthesis.

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Transparency document

The Transparency document associated with this article can be found, in the online version.

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