Biochimica et Biophysica Acta 1853 (2015) 1693–1701

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Protein kinase CK2 potentiates translation efficiency by phosphorylating eIF3j at Ser127

Christian Borgo [a,b], Cinzia Franchin [c], Valentina Salizzato [a,b], Luca Cesaro [a,b], Giorgio Arrigoni [c], Laura Matricardi [d], Lorenzo A. Pinna [a,b], Arianna Donella-Deana [a,b,*]

[a] Department of Biomedical Sciences, University of Padova, Via U. Bassi 58B, 35131 Padova, Italy
[b] CNR Institute of NeuroSciences, University of Padova, Via U. Bassi 58B, 35131 Padova, Italy
[c] Proteomic Center of Padova University, Via G. Orus B2, 35129 Padova, Italy
[d] Venitian Institute of Oncology (IOV-IRCCS), Via Gattamelata 64, 35128 Padova, Italy

ARTICLE INFO

Article history:
Received 18 December 2014
Received in revised form 17 March 2015
Accepted 7 April 2015
Available online 15 April 2015

Keywords:
eIF3j phosphorylation
Protein kinase CK2
eIF3 complex
eIF3 assembly
Translation initiation activation

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 j-subunit 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.

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 IF3a to eIF3m in order of decreasing molecular weight, and possesses an anthropomorphic 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

* Corresponding author at: Department of Biomedical Sciences, University of Padova, Via U. Bassi 58B, 35131 Padova, Italy. Tel.: +39 049 8276110; fax: +39 049 8276363.
E-mail address: arianna.donella@unipd.it (A. Donella-Deana).

http://dx.doi.org/10.1016/j.bbamcr.2015.04.004
0167-4889/

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 eIF3 complex. CK2-catalyzed phosphorylation of eIF3j triggers the association of this subunit with eIF3 complex promoting an efficient translation initiation.

2. Materials and methods

2.1. Materials and antibodies

[γ-^{33}P]ATP was purchased from Hartmann Analytic GmbH (Braunschweig Germany). Protease inhibitor cocktail was from Calbiochem (Darmstadt, Germany), while phosphatase inhibitor cocktails 2 and 3 and the kit for molecular weight markers were from Sigma-Aldrich (Dorset, UK). CX-4945 was purchased from AbMole BioScience (Hong Kong, China), staurosporine from Sigma-Aldrich (Dorset, UK) and quinalizarin was kindly provided by Dr. G. Cozza, University of Padova, Italy [20]. RRRADDSDDDDD peptide [21] and recombinant CK2 ($\alpha_2\beta_2$) [22] were kindly provided by Dr. Oriano Marin and Dr. Andrea Venerando (University of Padova, Italy), respectively. Antiserum against CK2α subunit was raised in rabbit using the peptide reproducing the sequence of the human catalytic subunit at the C-terminus (376–391). The peptide was coupled to keyhole limpet hemocyanin using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (Fischer Scientific, Illkirch Cedex, France). Anti-CK2β antibody was from Epitomics (Burlingame, CA), while antibodies raised against Fyn, eIF3j, eIF3b, eIF3c, eIF3d, and rpS6 were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Akt1 (phospho-129) was from Abcam (Cambridge, UK), anti-Akt1 from Cell Signaling Technology (Danvers, MA), while anti-β-actin and anti-c-Myc were purchased from Sigma-Aldrich.

2.2. Cell culture

HEK293 (human embryonic kidney) and Hela (cervical cancer) cells were grown in DMEM, while LAMA84 (chronic myeloid leukemia) cells were maintained in RPMI 1640. Both media were supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin.

2.3. 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).

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-eIF3j and anti-Myc immunoprecipitates were phosphorylated in 25 μl of a phosphorylation medium containing 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl and 20 μM [γ-^{33}P]ATP (about 1000 c.p.m./pmol). Samples were subjected to SDS-PAGE and blotted. Radioactive proteins were evidenced by a Cyclone Storage Phosphor-Screen (PerkinElmer, Waltham, MA).

2.6. Identification of the eIF3j site phosphorylated by CK2

HEK293 cells, treated with vehicle or CX-4945 were immunoprecipitated with eIF3j 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 home-made TiO_2 micro-columns (prepared as described in [23]) preconditioned 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 NH_4OH (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 (10×10^6) were lysed with the lysis buffer described in Section 2.3 containing 0.2% Triton X-100 and 10 mM KCl (instead of 1% Triton X-100 and 150 mM NaCl, respectively). Lysates (300 μl containing 400 μg of proteins) were layered on the top of a 3.6 ml of a glycerol discontinuous gradient (0.9 ml of 40%, 0.9 ml of 30%, 0.9 ml of 20% and 0.9 ml of 10%) in 50 mM Hepes, pH 8.0, 1 mM EDTA, 1 mM DTT, protease and phosphatase inhibitors. The tubes were centrifuged in a Beckman SW60Ti rotor at 100,000 × *g* for 18 h at 4 °C and fractionated from the bottom into 19 fractions. Fractions (40 μl) were analyzed by western blot.

2.8. RNA interference

HEK293 cells (5×10^5) were transfected for 72 h with 30 nM CK2α and CK2β specific siGENOME SMARTpool siRNAs (Dharmacon, Lafayette, CO, USA) or non-specific siRNA siCONTROL riscfree#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 $MgCl_2$, 400 μM synthetic peptide-substrate RRRADDSDDDDD and 20 μM [γ-^{33}P]ATP (1000 cpm/pmol). Assays