Biochimica et Biophysica Acta 1830 (2013) 2938–2945

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

CK2 phosphorylation of human Sec63 regulates its interaction with Sec62

Emmanuel Ampofo, Sabrina Welker, Martin Jung, Linda Müller, Markus Greiner, Richard Zimmermann, Mathias Montenarh *

Medizinische Biochemie und Molekularbiologie und Kompetenzzentrum Molekulare Medizin (KOMM), Universität des Saarlandes, Gebäude 44, 66424 Homburg, Germany

ARTICLE INFO

Article history:
Received 17 July 2012
Received in revised form 17 December 2012
Accepted 19 December 2012
Available online 31 December 2012

Keywords:
Protein kinase
Phosphorylation
Protein–protein interaction
Protein translocation

ABSTRACT

Background: Protein kinase CK2 is a pleiotropic enzyme which is ubiquitously expressed in eukaryotic cells. Several years ago CK2 was found to be associated with the mammalian endoplasmic reticulum. So far nothing is known about the function of CK2 at the ER.
Methods: CK2 phosphorylation sites in the polypeptide chain of Sec63 were mapped using deletion mutants and a peptide library. Binding of Sec63 to CK2 and to Sec62 was analyzed by pull-down assays and by co-immunoprecipitation
Results: Sec63 was identified as a novel substrate and binding partner of protein kinase CK2.
We identified serine 574, serine 576 and serine 748 as CK2 phosphorylation sites. Phosphorylation of Sec63 by CK2 enhanced its binding to Sec62.
Conclusions: Protein kinase CK2 phosphorylation of Sec63 leads to an enhanced binding of Sec63 to Sec62. This complex formation is a prerequisite for a functional ER protein translocon.
General significance: Thus, our present data indicate a regulatory role of CK2 in the ER protein translocation.

1. Introduction

In eukaryotic cells protein translocation across the membrane of the endoplasmic reticulum is the first step for the correct localization of membrane proteins and soluble proteins into the secretory pathway [1]. The transport of proteins with a signal sequence across the ER membrane is accomplished by the Sec61 complex which forms a channel in the ER membrane. In yeast, Sec61p assembles with Sec62p and Sec63p to form the functional unit for post-translational protein translocation [2]. Sec62p recognizes the signal-sequence, thus targeting the newly synthesized protein to the translocation channel in the ER membrane. In mammalian cells in addition to a Sec62 dependent translocation there are also indications for a Sec62 independent translocation. The Sec62 dependent mechanism is required in particular for small polypeptides [3]. In yeast the N-terminus of Sec62p directly interacts with the C-terminal end of Sec63p. This interaction is conserved in mammals and the two proteins are involved in the translocation of certain precursor polypeptides into the mammalian ER [4]. Sec63 belongs to a family of DNA J proteins with three membrane-spanning domains [5] (Fig. 1). Its J-domain is located between the transmembrane regions two and three in the lumen of the ER where it interacts with the ER located Hsp70 protein BiP.

Modulating the activity of proteins is often achieved by post-translational modifications including phosphorylation. More than ten years ago, protein kinase CK2 was shown to be associated with the mammalian ER [6]. Protein kinase CK2 is a ubiquitously expressed protein kinase which is composed of two catalytic α- or α′-subunits and two non-catalytic β-subunits [7]. Several reports have shown that life without CK2α and CK2β is impossible. Mice lacking CK2α′ are viable but male mice are infertile. The important role of CK2 for viability of eukaryotic cells is further supported by the large number of substrates whose activities are regulated by CK2 phosphorylation [8]. One of the ER associated proteins that are a substrate for CK2 is the J-domain containing protein ERj1 [9]. Like Sec63, ERj1 also contains a luminal J-domain [10] (Fig. 1). Furthermore, expression of human ERj1 in yeast can complement an inactivation of the Sec63 gene [11]. It was already shown that the Ca^{2+}-dependent isoform of protein kinase C is associated with rough microsomes from dog pancreas [12]. Moreover, it was shown that protein kinase CK2 phosphorylated several proteins from puromycin/high-salt-treated rough microsomes. However, these proteins were not further characterized. It was shown that the Sec63p from *Saccharomyces cerevisiae* is phosphorylated by CK2 [13].

So far, it is not clear whether the mammalian Sec63 is also phosphorylated by CK2. Therefore, we addressed this question in the present paper. We also attempted to localize the phosphorylation site on the polypeptide chain of human Sec63 and we analyzed the functional role of this phosphorylation for the interaction of Sec63 with Sec62. We found that Sec63 bound to the CK2 holoenzyme and Sec63 is phosphorylated by the CK2 holoenzyme at serine 574, serine 576 and serine 748 *in vitro*. Our results further show that the phosphorylation of Sec63 increases the binding of Sec62 *in vitro*.

* Corresponding author. Tel.: +49 6841 162 6502; fax: +49 6841 162 6027.
E-mail address: Mathias.Montenarh@uks.eu (M. Montenarh).

0304-4165/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2012.12.020

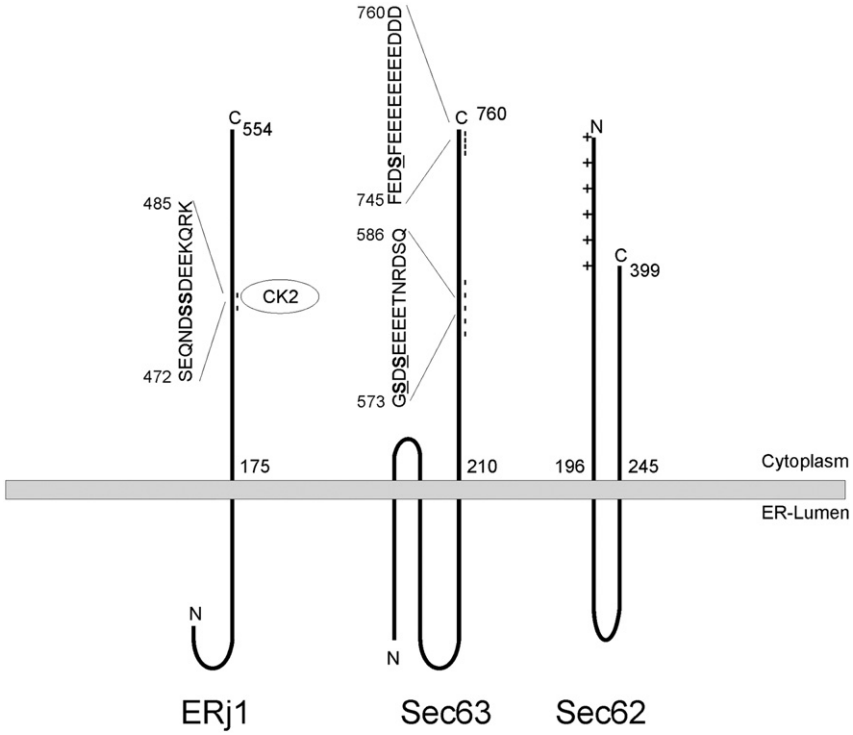

Fig 1. Schematic illustration of human Sec63, ERj1 and Sec62.

2. Materials and methods

2.1. Chemicals and biological reagents

Tissue culture media were purchased from GIBCO. The foetal calf serum (FCS) was from PAA (Pasching, Austria) and the [^{32}P]γATP from Hartmann Analytic (Braunschweig, Germany). 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI) was from Roche (Mannheim, Germany) and CHAPS was purchased from Calbiochem, Bad Soden. The CK2 specific inhibitor CX4945 was purchased from Selleckchem (Munich, Germany).

2.2. Cell lines

HepG2 cells (ATCC; HB-8065) were cultured in Dulbecco's modified Eagle's medium containing 10% foetal calf serum (FCS) and 1% glutamine. Cells were cultured at 37 °C and 5% CO_2 in a humidified atmosphere in an incubator.

2.3. Plasmid DNA constructs

Deletion mutants of Sec63 were created by PCR and inserted into the plasmid pGEX-4T-1 in frame with the GST-coding sequence as described in [14]. Sec62N-His was generated as described in [15]. All new constructs were sequenced before use.

2.4. Antibodies

The monoclonal antibodies 1A5 (anti-CK2α) and 6D5 (anti-CK2β) were previously described [16,17]. Goat anti-mouse IgG (No. 115-035-146) and goat anti-rabbit IgG (No. 111-035-144) were from Dianova (Hamburg, Germany). Anti-Sec63 antibody is a rabbit antibody directed against recombinant Sec63C protein.

2.5. Co-immunoprecipitation

HepG2 cells were lysed with RIPA buffer containing 1% NP40 and 0.65% CHAPS. After lysis cell debris was removed by centrifugation at 13,000 ×*g*. The protein content was determined with the BioRad reagent dye (BioRad, München, Germany). For co-immunoprecipitation, cell lysates were pre-cleared twice with a mixture of protein A agarose beads and CL 4-B agarose beads over a period of 45 min. The supernatant was incubated with a Sec63 specific antibody (15 μl) for 90 min. Beads were washed twice with PBS, pH 7.4. Bound proteins were eluted with SDS sample buffer (195 mM Tris/HCl, pH 6.8, 6% SDS, 15% β-mercaptoethanol, 30% glycerol, 0.03% bromophenol blue) and separated on a 12.5% SDS polyacrylamide gel, transferred to a PVDF membrane and analyzed by Western blot with specific antibodies.

2.6. Purification of recombinant proteins

The recombinant GST-tagged proteins were purified as described in [14], the recombinant His-tagged proteins were purified as described in [15].

2.7. Pull-down assay

Purified GST proteins (10 μg) were immobilized on GSH-sepharose and incubated with His-tagged Sec62 in a buffer (20 mM HEPES–KOH, pH 7.5, 150 mM KCl, 1 mM EDTA, 1.5 mM $MgCl_2$, 0.65% CHAPS). The pull-down reaction was carried out with 15 μg of recombinant CK2 holoenzyme. After washing, bound proteins were eluted and analyzed by SDS polyacrylamide gel electrophoresis, followed by protein staining with Coomassie Brilliant Blue.

2.8. In vitro phosphorylation with protein kinase CK2

Recombinant GST-tagged Sec63C proteins were mixed with equal amounts of CK2 holoenzyme in a volume of 25 μl of kinase buffer (50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 50 μM ATP,