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Eukaryotic ribosomes host PKC activity

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ABSTRACT

PKC isoform βII modulates translation and can be recruited on ribosomes via its scaffold RACK1 (receptor for activated protein kinase C 1), which resides on the 40S ribosomal subunit. However, whether a PKC activity exists on the ribosome is not yet demonstrated. We purified native ribosomes by two different techniques, which avoid stripping of initiation factors and other associated proteins. In both cases, purified ribosomes are able to phosphorylate a specific PKC substrate, MARCKS (Myristoylated Alanine-Rich C-Kinase Substrate). MARCKS phosphorylation is switched on by treatment with PKC agonist PMA (Phorbol 12-Myristate 13-Acetate). Consistently, the broad PKC inhibitor BMI (Bisindolyl Maleimide I) abrogates MARCKS phosphorylation. These data show that native ribosomes host active PKC and hence allow the phosphorylation of ribosome-associated substrates like initiation factors and mRNA binding proteins.

The protein kinase C (PKC) superfamily entails 11 isoforms involved in most cellular processes [1]. PKCs are divided into three groups: classical or conventional PKCs (cPKCs), novel PKCs (nPKCs), and atypical PKCs (aPKCs) [2]. Conventional PKCs (PKCα, PKCβI/II, PKCγ) require both diacylglycerol (DAG) and Ca^{2+} for activation. They can also be activated by phorbol esthers, such as Phorbol 12-Myristate 13-Acetate (PMA), in a Ca^{2+}-dependent manner. Novel PKCs (PKCδ, PKCε, PKCη, PKCθ, PKCσ) are DAG/PMA sensitive, but do not respond to Ca^{2+}, whereas atypical PKCs (PKCζ and PKCι/λ) are unresponsive both to DAG and Ca^{2+} [3]. It has been proposed that each PKC isoform has its specific scaffold protein, namely Receptor for Activated C Kinase or RACK [4]. RACK1 is an anchoring proteins for PKCβII [5]. Interestingly, RACK1 is present on 40S ribosomes [6], and on mRNPs particles [7]. RACK1 binds also eukaryotic initiation factor 6 (eIF6), and in vitro mediates its release from the 60S ribosome in the presence of PKC [8]. A cryo-EM map of the eukaryotic ribosome showed that RACK1 is located in proximity of the mRNA exit channel, in close contact with the binding surface of eIF3 complex, next to mRNA binding proteins [9]. These data suggested that RACK1 may function as a PKC adaptor protein on the ribosomal subunit [10]. Indeed, we recently reported that PKCβII isoform can be translocated to RACK1 containing ribosomes and increase the rate of translation [11]. However, this study left unanswered the question on whether active PKC can be actually present on ribosomes.

In this study, by means of two different techniques, we purified ribosomes in native conditions in order to preserve also loosely associated factors. We found that in both cases, these ribosomes exhibited a PKC-like activity, being able to phosphorylate MARCKS, a specific PKC substrate. This is the first evidence showing an active kinase associated with ribosomes.

Materials and methods

Cell lines and reagents. HEK293 and HeLa cells were cultured in DMEM medium (Gibco) supplemented with 10% FBS (Gibco), glutamine, and antibiotics, according to standard procedures. Transfections were carried out using either calcium phosphate standard protocol [12] or Gene Porter Transfection Reagent (GTS) according to manufacturer instruction. PKCβII plasmid was from A. Newton lab (University of California at San Diego, La Jolla, California, USA) [13]. GST-MARCKS was a kind gift of J.-W. Soh (Inha University, Incheon, Korea) [14]. Constructs GFP-S18 and GFP-L5 were

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prepared by subcloning full length rpS18 and rpL5 ribosomal proteins in pEGFP-N2 upstream of Enhanced GFP (Clontech). Autofluorescence of cells expressing GFP fusion proteins were acquired with a Zeiss Axiophot equipped with 63X immersion lenses. PMA (phorbol 12-myristate 13-acetate), cycloheximide, and proteases inhibitor cocktail were from Sigma. The PKC inhibitor BMI (bisindolyl maleimide I) and BSA (bovine serum albumin) were from Calbiochem. RNasin® was from Promega. Cells were stimulated with 50 nM PMA for 30 min, or pre-treated with 100 nM BMI, 30 min before stimulation.

Ribosomes purification and polysomal profiles. HEK293 cells were treated 10 min with 100 μg/ml cycloheximide, washed in PBS, and then lysed in buffer A [50 mM Tris–HCl, pH 7.4, 100 mM NaCl, 30 mM $MgCl_2$, 0.1% Igepal, 100 μg/ml cycloheximide, 40 U/ml RNasin®, protease inhibitor cocktail]. Whole cell extracts were clarified at 4 °C, 10 min at 15,000*g*. Extracts were loaded on a 30% sucrose cushion in 50 mM Tris–acetate, pH 7.5, 50 mM NH_4Cl, 12 mM $MgCl_2$, and 1 mM DTT and ultracentrifuged overnight in a SW41Ti Beckman rotor at 39,000 rpm. The ribosomal pellet was resuspended in buffer B [50 mM HEPES–NaOH, pH 7.35, 100 mM $MgCl_2$, 10 mM $CaCl_2$, 1 mM DTT, 2 mM EGTA, 1 mM NaF, 10 mM β-glycerophosphate]. Polysomal profiles were performed as previously described [11].

Western blot analysis and antibodies. Proteins were separated on SDS–PAGE, transferred to Immobilon-P membranes (Millipore), and probed with appropriate antibodies. The following antibodies were used: rabbit polyclonal anti-rpS6 (Cell Signaling); goat anti rpL28 (A-16) (Santa Cruz); mouse monoclonals anti-RACK1, anti-PKCβ, anti-PKCι, anti-PKCε (BD Transduction Laboratories); rabbit polyclonal isoform-specific anti-PKCβII (C-18) (Santa Cruz); mouse monoclonal anti-GFP (Roche). Rabbit polyclonal anti-eIF6 (p27BBP) was previously described [15]. Rabbit polyclonal anti-GST was a kind gift of Dr. Massimilano Pagani (Primm s.r.l., Milano, Italy).

Kinase assay. Recombinant GST-MARCKS protein expression was induced in *Escherichia coli* strain BL21 and purified using Glutathione Sepharose 4B (Pharmacia Biotech) according to standard procedures.

The kinase assay from ribosomes purified by ultracentrifugation was performed as follows. Thirty absorbance units at 254 nm of ribosomes from HEK293 cells resuspended in buffer B were added to 10 μg GST-MARCKS and 10 μCi γ-^{32}P-ATP. The reaction was run at 30 °C for 30 min and terminated by adding one volume of sample buffer. The samples were boiled 5 min, separated by SDS–PAGE and stained with Coomassie brilliant blue R-250. Autoradiography was performed on dried gel.

The kinase assay from GFP-pulldown ribosomes was performed as described [14], with little modifications. HeLa cells were transfected with the indicated expression plasmids and immunoprecipitation was performed as described below. The immunoprecipitates were added to the kinase assay with 10 μg GST-MARCKS substrate and 5 μCi of γ-^{32}P-ATP. The reaction was performed at 30 °C for 1 h and terminated by adding one volume of sample buffer. The samples were boiled 5 min and separated by SDS–PAGE and stained with Coomassie brilliant blue R-250. Autoradiography was performed on dried gel.

Immunoprecipitation. Adherent, subconfluent HeLa cells were lysed in buffer C [50 mM HEPES pH 7.5, 150 mM NaCl, 0.1% Tween 20, 1 mM EDTA, 2.5 mM EGTA, 10% glycerol, 1 mM NaF, 0.1 mM Na_3VO_4, 10 mM β-glycerophosphate, proteases inhibitor cocktail]. The whole cell extract was clarified at 4 °C, 15,000*g*, 10 min. The amount of protein recovered was quantified by the BiCinchonic Acid (BCA) protein assay (Pierce). For immunoprecipitation, 3 mg total proteins were pre-cleared with protein G beads (Amersham), and then incubated for 2 h at 4 °C with 2 μg/ml mouse monoclonal anti-GFP or with an irrelevant antibody. The kinase assay was performed as above. Protein G beads were added and incubated for 1 h at 4 °C. The beads were washed four times with lysis buffer and resuspended in sample buffer. Immunoprecipitates were resolved on SDS–PAGE, transferred to Immobilon-P membranes (Millipore), and probed with appropriate antibodies.

Results

Purified ribosomes from HEK293 cells host PKC kinase activity

We have recently shown that PKCβII can associate with mammalian ribosomes and may regulate translation upon binding to the ribosome-associated protein RACK1 [11]. However, PKC activity has not been detected on ribosomes yet, thus raising the possibility of presence of inactive PKC on ribosomes. We attempted to address this question.

As a first step, we purified native ribosomes, not stripped by initiation factors by high salts, to evaluate whether PKC activity could be detected. Total ribosomes purified from HEK293 cells on a sucrose cushion were resuspended avoiding salt stripping of loosely ribosome-associated factors and dissociation of mature translating subunits. Purified ribosomes correctly had structural protein rpS6 on 40S–80S fractions, and rpL28 on 60S–80S. In these conditions, the scaffold protein RACK1, which is associated with 40S subunits and is located close to the mRNA exit channel of mature 80S [9] is retained on the ribosome and partially cosediments with eIF6 (Fig. 1A) as expected [8].

PMA is widely used to activate classical and novel PKC isoforms. In order to scout for the presence of PKC activity on the ribosome, we used PMA treatment to induce kinase activation. Ribosomes from PMA stimulated or unstimulated cells were purified as above and mixed in vitro, with γ-^{32}P-ATP and Myristoylated Alanine-Rich C-Kinase Substrate protein (MARCKS) [16]. GST-MARCKS is a well-defined synthetic PKC substrate [17]. Ribosomes from PMA treated cells can phosphorylate GST-MARCKS (Fig 1B), suggesting a PMA dependent, PKC-like activity hosted on purified ribosomes. To establish whether the phosphorylation of GST-MARCKS is due to

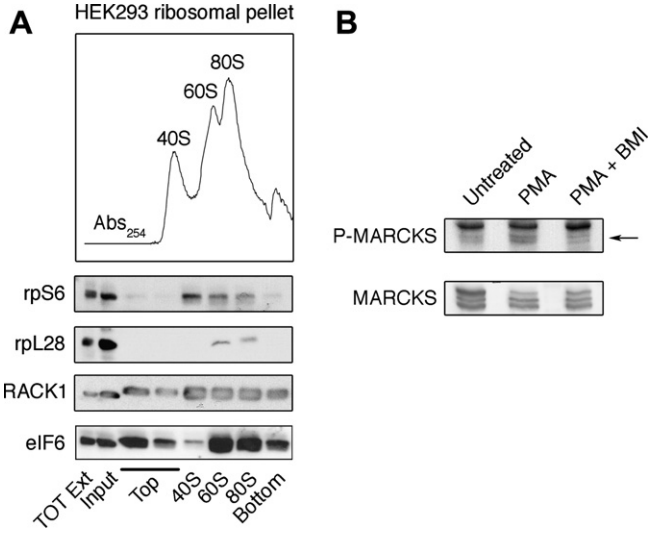

Fig. 1. Ribosomes host a PKC activity. (A) HEK293 cells extract (TOT Ext) was ultracentrifuged on sucrose cushion to pellet ribosomes. The resuspended ribosomal pellet (Input) was further separated on sucrose gradient. 40S, 60S, and 80S are observed. Fractions were collected, TCA concentrated and analyzed by SDS–PAGE. Proteins were probed for rpS6, rpL28, RACK1, and eIF6. (B) Ribosomes were prepared as in (A) from untreated or PMA stimulated HEK293 cells and incubated with the PKC substrate GST-MARCKS in presence of γ-^{32}P-ATP. Broad PKC inhibitor BMI was used where indicated. Proteins were separated by SDS–PAGE and phospho-proteins were revealed by autoradiography. Anti-GST shows equal loading of kinase substrate. The arrow points to the specific MARCKS band.

the copurification of a PKC isoform, we pre-treated cells with PKC inhibitor BMI (Bisindolyl Maleimide I) [18]. Ribosomes from BMI treated cells showed reduced phosphorylation of GST-MARCKS, as compared to PMA stimulated cells (Fig 1B). Thus, PKC activity copurifies with native ribosomes.

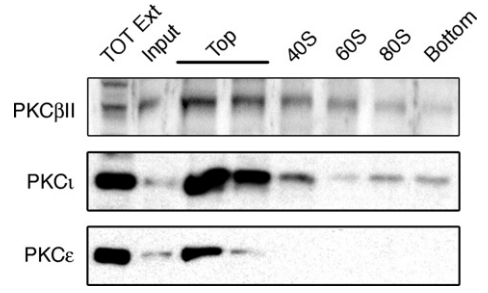

Fig. 2. Ribosomes were purified and separated on sucrose gradient as in Fig. 1A. Fractions were TCA concentrated and analyzed by SDS–PAGE. Protein cosedimenting with ribosomes were probed for the presence of PKC isoforms. PKCβII and PKCι were found to cosediment with ribosomes. PKCε is present in the purified ribosomes input, but does not associates with ribosomes.

Our previous work has shown that PKCβII can bind RACK1 and associate with ribosomes, *in vivo*. Here we analysed which PKC isoforms are associated with purified ribosomes. Ribosome suspension was separated on sucrose gradient and fractions analyzed for PKC content (Fig. 2 and data not shown). Classical PKC isoform βII and atypical isoform ι were found to cosediment with ribosomal subunits. PKCι is a PMA insensitive kinase [1] not involved in protein synthesis regulation [11], and was not further investigated. Association of PKCβII with ribosomes is specific, since PKCε, which also binds RACK1 [19], is absent from ribosomal fractions. We further investigated the activity of PKCβII isoform on ribosomes.

GFP-tagged ribosomes copurify PKC kinase activity

We previously showed that transfection of exogenous PKCβII in HeLa cells led to association of this kinase with ribosomes, and an increase in translation [11]. In order to gather independent evidence on the association of PKCβII to ribosomes we turned to HeLa cells. We also switched to a different technique of purification, which is immunoprecipitation of GFP-tagged ribosomes.

Two constructs carrying the EGFP cassette fused to the C-terminus of rpS18 and rpL5 were developed, GFP-S18 and GFP-L5

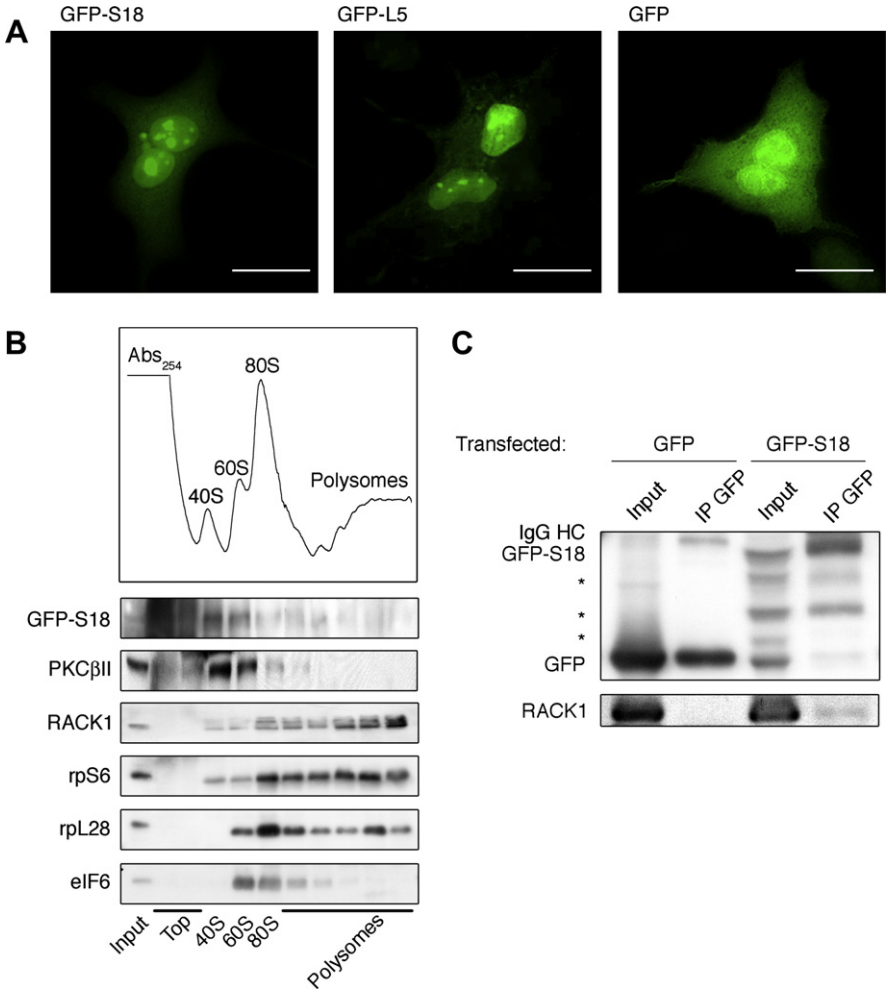

Fig. 3. Generation of cells with GFP-tagged ribosomes (A) HeLa cells are transfected with either GFP-S18 or GFP-L5 or GFP expression constructs. Autofluorescence is observed 48 h after transfection. Scale bar, 20 μm. Nucleolar and cytosolic localization of GFP fusion proteins is observed. (B) Polysomal profile of GFP-S18 transfected cells was performed. Cellular rate of translation is not impaired by GFP fusion protein. Factions were collected, TCA concentrated and separated by SDS–PAGE. GFP-S18 cosediments through the sucrose gradient. Ribosome markers rpS6 and rpL28 confirm ribosome localization. PKCβII, the scaffold protein RACK1 and eIF6 cosediment as expected. (C) Anti-GFP immunoprecipitation is carried either on GFP or GFP-S18 transfected cells. Pull-downs were probed with either anti-GFP or with anti-RACK1 as a marker for the ribosome. RACK1 is incorporated in GFP-S18 containing ribosomes.

(Fig. 3). HeLa cells were transfected with either GFP-S18 or GFP-L5 or control GFP protein alone. GFP-S18 accumulated in the nucleolus, where ribosomes are assembled, and in the cytosol; similar results were obtained for GFP-L5, but not for GFP alone, which never targeted to nucleoli (Fig. 3A).

To confirm incorporation of GFP-fusion protein into the translating machinery, ribosomes were separated on sucrose gradient and fractions were analyzed by immunoblotting. Polysomal profile of GFP-S18 transfected cells show defined polysomes, indicating that the exogenous protein does not impair translation (Fig. 3B top panel). GFP-S18 is present through the gradient and it cosediments in fractions containing 40S subunits, 80S mature ribosomes and translating ribosomes, as indicated by ribosomal markers rpS6 and rpL28 (Fig 3B bottom panels). The difference in the distribution between endogenous ribosomal rpS6 and transfected GFP-ribosomal proteins is likely due to the fact that excess ribosomal proteins not incorporated on ribosomes remain in the soluble phase or are degraded (see also section Discussion).

RACK1 is present only on cytoplasmic ribosomes [8]. In order to prove that at least part of GFP-ribosomes were cytosolic, and involved in protein synthesis, we tested whether ribosomes incorporating GFP-S18 also contained RACK1. HeLa cells were either transfected with GFP-S18 or were mock transfected. GFP immunprecipitation resulted in enrichment of GFP-S18 fusion protein. Immunoprecipitation of GFP-S18 resulted in copurification of endogenous RACK1 in cells transfected with GFP-S18, whereas control GFP alone does not coprecipitate with RACK1 (Fig. 3C). Thus, GFP-S18 is recruited in RACK1 containing ribosomes. Degradation products of GFP-S18 were also observed, but importantly were not enriched in the immunoprecipitation.

Next, we tested whether ribosomes pulled down with anti-GFP were able to phosphorylate MARCKS. Cells were transfected with GFP-S18, GFP-L5 or control GFP alone and PKC was PMA-activated. The immunoprecipitates were employed to phosphorylate GST-MARCKS, *in vitro*, in the presence of γ-^{32}P-ATP. Data show that immunoprecipitation of GFP-S18 and GFP-L5 result in the phosphorylation of GST-MARCKS (Fig. 4A), while control GFP does not copurify any PKC activity against the specific substrate. Control experiments showed also that 1- the kinase activity is specifically associated to GFP immunoprecipitation and not aspecifically purified by agarose beads (Fig. 4B), 2-phosphorylation of GST-MARCKS is PMA dependent, and does not occur when PKC is inactivated (Fig. 4C).

We conclude that a PKC activity can associate with translating ribosomes.

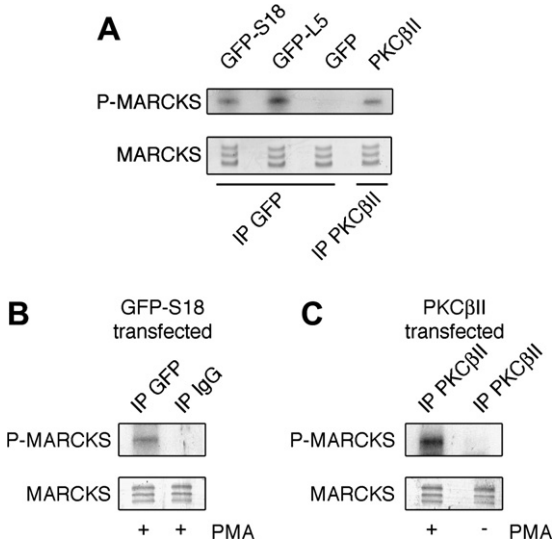

Fig. 4. GFP-ribosomes pull-down PKC activity. (A) HeLa cells are co-transfected with either GFP-S18, or GFP-L5 or GFP alone together with PKCβII as indicated. 48 h after transfection, cells are PMA stimulated and lysed. Anti-GFP immunoprecipitation is performed in order to pull-down GFP-ribosomes. Immunopurified ribosomes are incubated with GST-MARCKS in presence of γ-^{32}P-ATP. Proteins are separated on SDS–PAGE and stained with Coomassie Blue. Autoradiography is performed on dried gel. PKCβII immunoprecipitate is a positive control for GST-MARCKS phosphorylation. (B) GFP-S18 transfected cells were immunoprecipitated with anti-GFP or with irrelevant antibody as a control. Kinase assay on GST-MARCKS was performed as above. PKC activity is specifically purified by immunoprecipitation. (C) Cells were treated with PMA to induce PKCβII activation or left unstimulated. Immunoprecipitation of PKCβII was used in kinase assays to phosphorylate the substrate as above. GST-MARCKS phosphorylation is PMA dependent.

Discussion

We collect evidence that a PKC activity resides on the ribosome, supporting the model of a physical contact between the kinase and the translational machinery.

In this study, we purified ribosomes in order to measure PKC activity associated with translational apparatus. Purifying ribosomes from mammalian cells in native conditions is demanding, since the translational machinery is a huge complex of structural proteins, regulating factors and different RNAs of about 4.2 MDa. In order to measure kinase activities associated to ribosomes, we need to maintain both weak and strong associations. Some reports in literature exist, mostly based on sucrose gradient sedimentation (see for example [20]). However, these procedures yield ribosomes stripped from non-structural ribosomal proteins. In the present study, we attempted to preserve associated factors by using physiological salt concentrations.

To further demonstrate that PKC association with ribosomes was not due to a purification artifact, we attempted to obtain similar results with a completely different technique, based on GFP-pulldown purification. We searched for ribosomal proteins exposed on the ribosome surface, that could be accessible from the soluble phase. rpS18 and rpL5 reside on the solvent phase of the ribosome [21]. GFP fusion constructs were prepared and correct internalization in translating ribosomes was confirmed (Fig. 3). Due to competition with endogenous ribosomal proteins, some of GFP-fusion proteins were degraded. This is expected since ribosomal proteins not incorporated into ribosomes are unstable. However, the purification by GFP immunoprecipitation resulted in the enrichment of correctly assembled ribosomes containing RACK1. Ribosomes obtained by GFP immunoprecipitation also exhibited a PKC activity, which was dependent by PMA.

Another important question to be addressed refers to the physiological substrates of PKC that act on the translational machinery. The RACK1–PKC complex is able to mediate the release of antiassociation factor eIF6 (eukaryotic initiation factor 6) from the large ribosomal subunit, allowing the joining of ribosomes *in vitro* [8]. Later studies confirmed that a PKC stimulated pathway results in the phosphorylation of eIF6, coincident with an increase of translation [22], and eIF6 itself may regulate global translation depending on the Ser235 PKC consensus site (Gandin et al., in press). However, eIF6 may be not the only substrate. Other factors associated to the ribosomal machinery have potential consensus sites for PKC phosphorylation. *In vitro* studies have shown that some of them can be indeed efficiently phosphorylated, like eIF4E, eIF4G [23], and eEF2 [24], but their physiological relevance is unclear. eIF4B, a stimulator of eIF4A helicase activity also contains a PKC consensus site which is phosphorylated *in vivo* [25,26]. In conclusion, multiple targets, in different cells may be responsible for the effects of PKC on translation.

In conclusion, the size and structure of the ribosomal complex may have evolved to dock signaling enzymes. Intriguingly, recent

work has extended the concept of a ribosomal kinase platform showing that the large initiation factor complex eIF3 can itself recruit S6K1 and mTOR [27]. Active PKCβII may also act on ribosomes, contributing to the ideas that the ribosomal machinery can act as a local transducer of translation on selected ribosomes.

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References

[1] H. Mellor, P.J. Parker, The extended protein kinase C superfamily, Biochem. J. 332 (Pt 2) (1998) 281–292.
[2] P.J. Parker, J. Murray-Rust, PKC at a glance, J. Cell Sci. 117 (2004) 131–132.
[3] H.J. Mackay, C.J. Twelves, Targeting the protein kinase C family: are we there yet?, Nat Rev. Cancer 7 (2007) 554–562.
[4] V. Kheifets, D. Mochly-Rosen, Insight into intra- and inter-molecular interactions of PKC: design of specific modulators of kinase function, Pharmacol. Res. 55 (2007) 467–476.
[5] D. Ron, C.H. Chen, J. Caldwell, L. Jamieson, E. Orr, D. Mochly-Rosen, Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins, Proc. Natl. Acad. Sci. USA 91 (1994) 839–843.
[6] A.J. Link, J. Eng, D.M. Schieltz, E. Carmack, G.J. Mize, D.R. Morris, B.M. Garvik, J.R. Yates 3rd, Direct analysis of protein complexes using mass spectrometry, Nat. Biotechnol. 17 (1999) 676–682.
[7] F. Angenstein, A.M. Evans, R.E. Settlage, S.T. Moran, S.C. Ling, A.Y. Klintsova, J. Shabanowitz, D.F. Hunt, W.T. Greenough, A receptor for activated C kinase is part of messenger ribonucleoprotein complexes associated with polyA-mRNAs in neurons, J. Neurosci. 22 (2002) 8827–8837.
[8] M. Ceci, C. Gaviraghi, C. Gorrini, L.A. Sala, N. Offenhauser, P.C. Marchisio, S. Biffo, Release of eIF6 (p27BBP) from the 60S subunit allows 80S ribosome assembly, Nature 426 (2003) 579–584.
[9] J. Sengupta, J. Nilsson, R. Gursky, C.M. Spahn, P. Nissen, J. Frank, Identification of the versatile scaffold protein RACK1 on the eukaryotic ribosome by cryo-EM, Nat. Struct. Mol. Biol. 11 (2004) 957–962.
[10] J. Nilsson, J. Sengupta, J. Frank, P. Nissen, Regulation of eukaryotic translation by the RACK1 protein: a platform for signalling molecules on the ribosome, EMBO Rep. 5 (2004) 1137–1141.
[11] S. Grosso, V. Volta, L.A. Sala, M. Vietri, P.C. Marchisio, D. Ron, S. Biffo, PKCbetaII modulates translation independently from mTOR and through RACK1, Biochem. J. (2008).
[12] F.L. Graham, A.J. van der Eb, Transformation of rat cells by DNA of human adenovirus 5, Virology 54 (1973) 536–539.
[13] A.S. Edwards, M.C. Faux, J.D. Scott, A.C. Newton, Carboxyl-terminal phosphorylation regulates the function and subcellular localization of protein kinase C betaII, J. Biol. Chem. 274 (1999) 6461–6468.
[14] J.W. Soh, E.H. Lee, R. Prywes, I.B. Weinstein, Novel roles of specific isoforms of protein kinase C in activation of the c-fos serum response element, Mol. Cell. Biol. 19 (1999) 1313–1324.
[15] S. Biffo, F. Sanvito, S. Costa, L. Preve, R. Pignatelli, L. Spinardi, P.C. Marchisio, Isolation of a novel beta4 integrin-binding protein (p27(BBP)) highly expressed in epithelial cells, J. Biol. Chem. 272 (1997) 30314–30321.
[16] A. Arbuzova, A.A. Schmitz, G. Vergeres, Cross-talk unfolded: MARCKS proteins, Biochem. J. 362 (2002) 1–12.
[17] J.W. Soh, I.B. Weinstein, Roles of specific isoforms of protein kinase C in the transcriptional control of cyclin D1 and related genes, J. Biol. Chem. 278 (2003) 34709–34716.
[18] D. Toullec, P. Pianetti, H. Coste, P. Bellevergue, T. Grand-Perret, M. Ajakane, B.V.P. Boissin, E. Boursier, F. Loriolle, The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C, J. Biol. Chem. 266 (1991) 15771–15781.
[19] A. Besson, T.L. Wilson, V.W. Yong, The anchoring protein RACK1 links protein kinase C epsilon to integrin beta chains. Requirements for adhesion and motility, J. Biol. Chem. 277 (2002) 22073–22084.
[20] A.V. Pisarev, A. Unbehaun, C.U. Hellen, T.V. Pestova, Assembly and analysis of eukaryotic translation initiation complexes, Methods Enzymol. 430 (2007) 147–177.
[21] C.M. Spahn, R. Beckmann, N. Eswar, P.A. Penczek, A. Sali, G. Blobel, J. Frank, Structure of the 80S ribosome from *Saccharomyces cerevisiae*-tRNA-ribosome and subunit–subunit interactions, Cell 107 (2001) 373–386.
[22] R. Carotenuto, N. De Marco, S. Biffo, M. Wilding, M.C. Vaccaro, P.C. Marchisio, T. Capriglione, G.L. Russo, C. Campanella, Phosphorylation of p27(BBP)/eIF6 and its association with the cytoskeleton are developmentally regulated in xenopus oogenesis, Cell. Mol. Life Sci. 62 (2005) 1641–1652.
[23] S.J. Morley, J.A. Traugh, Phorbol esters stimulate phosphorylation of eukaryotic initiation factors 3, 4B, and 4F, J. Biol. Chem. 264 (1989) 2401–2404.
[24] K. Kielbassa, H.J. Muller, H.E. Meyer, F. Marks, M. Gschwendt, Protein kinase C delta-specific phosphorylation of the elongation factor eEF-alpha and an eEF-1 alpha peptide at threonine 431, J. Biol. Chem. 270 (1995) 6156–6162.
[25] K. Moser, F.M. White, Phosphoproteomic analysis of rat liver by high capacity IMAC and LC-MS/MS, J. Proteome Res. 5 (2006) 98–104.
[26] D. Shahbazian, P.P. Roux, V. Mieulet, M.S. Cohen, B. Raught, J. Taunton, J.W. Hershey, J. Blenis, M. Pende, N. Sonenberg, The mTOR/PI3K and MAPK pathways converge on eIF4B to control its phosphorylation and activity, EMBO J. 25 (2006) 2781–2791.
[27] M.K. Holz, B.A. Ballif, S.P. Gygi, J. Blenis, mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events, Cell 123 (2005) 569–580.