SHORT COMMUNICATIONS

Regulation of S6K1 subcellular localization by Casein kinase 2

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Casein Kinase 2 (CK2) is physiological binding partner of ribosomal protein S6 kinase 1 identified using of two-hybrid yeast system. The specificity of interaction between в-subunit of CK2 and S6K1 was confirmed in vitro, also it was shown that Ser17 of S6K1 can be phosphorylated by CK2. The presented data suggest that Ser17 phosphorylation is the important event of S6K1 export from the nucleus. Immunoprecipitation studies of S6K1 and CK2 from cytoplasmic fraction of NIH 3T3 cells indicate the formation of protein complex S6K1/CK2 in the nucleus, but not in the cytoplasm. Further fluorescent microscopy studies demonstrated that phosphorylation mimicking mutant of S6K1(S17E) is not accumulated in the nucleus through the activated export of kinase from the nucleus.

Key words: *S6K1, S6K2, CK2в, nuclear export*

Introduction. The family of Ser/Thr protein kinases of 40S ribosome subunit S6 protein (S6K1 and S6K2) is one of the regulatory links in PI3K/mTOR signaling pathway, which is one of the main regulators of basic cell functions, namely cell growth, proliferation, differentiation and apoptosis. The S6K activity is regulated by multiple phosphorylation/dephosphorylation events induced by extracellular mitogenic stimuly [1]. Recently, we have identified new S6K1-binding protein, regulatory в-subunit of Casein kinase 2 (CK2в), by yeast two-hybrid system. The formation of the complex between CK2 and S6K1 was confirmed *in vitro*. Also, it was shown that CK2 can phosporylate Ser17 on S6K1 [2]. The further work was directed to confirm the existence of the *in vivo* complex and to determine the physiological significance of Ser17 phosphorylation.

Materials and methods. The mouse fibroblast cells (NIH3T3) were obtained from American Type Culture Collection (ATCC). Anti-CK2 б antibodies were from SantaCruz (USA); anti-lamin – from Cell Signalling (USA); anti-tubulin – from Sigma (USA). The production of antibodies to S6K1 was described earlier [3]. The NIH3T3 nuclear and cytoplasmic fractions extracts were prepared as described in [4]. Immunoblotting and immunoprecipitation were conducted according to the earlier developed method [5]. For immunofluorescent analysis, NIH3T3 cells were transiently trancfected by the

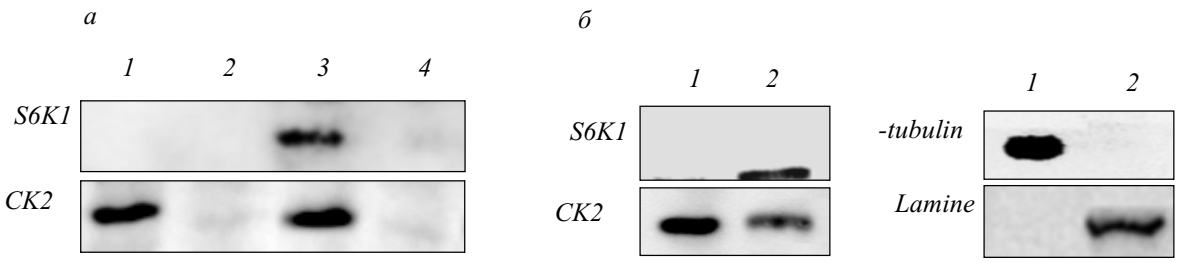

Fig.1. Western blot analysis of endogenous S6K1 and catalytic subunit of CK2 interaction in nuclear and cytoplasmic fractions of cells: a – Western blot analysis of immunoprecipitates with anti-CK2 antibodies from cytoplasmic (1) and nuclear (3) fractions of growing NIH3T3 cells with the use of anti-S6K1 and anti-CK2б antibodies; nonspecific binding of S6K1 and CK2 to protein A sepharose beads was tested in cytoplasmic (2) and nuclear (4) fractions; b – Western blot analysis of cytoplasmic (1) and nuclear (2) extracts of NIH3T3 cells with the use of anti-S6K1, anti-CK2б, anti-Lamin (marker of nuclear fraction) and anti-tubulin (marker of cytoplasmic fraction) antibodies.

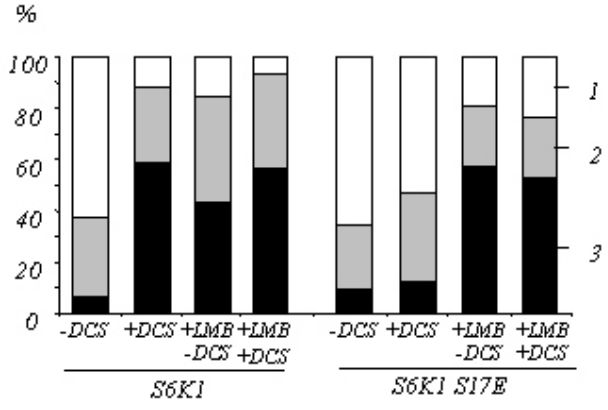

Fig.2. Analysis of subcellular localization of S6K1 wild type and S17E mutant by confocal microscopy. NIH3T3 cells transiently transfected by S6K1 wild type or S6K1 S17E mutant, expressed in frame with EE-tag, were starved without serum 24 hours. After that LMB (5nM) was added to marked samples and cells were additionally incubated for 12 hours, than cells were stimulated with 10% serum for 1 hour. Cells were fixed, probed with anti-EE antibody and fluorescein isothiocyanate-labeled anti-mouse immunoglobulin G, and analyzed by confocal microscopy. The number of cells showing nuclear (1), nuclear-cytoplasmic (2) or cytoplasmic (3) staining for each case was counted, and the percentage was calculated. At least 150 cells were counted for each condition.

appropriate plasmids, encoded S6K1, S6K S17E in frame with EE-tag epitope [6], using PolyFect reagent (QIAGEN, Great Britain), in accordance with manufacturer's recommendations. In 24 hours after transfection the cells were starved in the non-serum medium for 36 hours with further stimulation by addition of 10% serum. Leptomycin B (LMB) was added 10 hours before stimulation by serum. The cells were fixed and treated as described earlier [6], and monoclonal anti-EE-antibodies were used as primary antibodies. Confocal microscope Zeiss LSM510 was used to analyse fluorescent stained cells.

Results and discussions. CK2 is often involved in the regulation of subcellular localization of proteins through their phosphorylation, therefore we made the supposition regarding the role of Ser17 phosphorylation in regulation of S6K1 subcellular localization, minding the fact that Ser17 phosphorylation of S6K1 does not influence its activity. To determine subcellular localization of S6K1/CK2 complex on the level of endogenous proteins, nuclear and cytoplasmic extracts of NIH3T3 cells were analysed. Fig.1.a. shows that interaction of endogenous S6K1 and CK2 occurs only in the nucleus, regardless that both enzymes were detected in nucleus and in the cytoplasm (Fig.1.b). Thus, CK2-mediated Ser17 phosphorylation of S6K1 most likely takes place in the nucleus.

To determine the physiological role of Ser17 phosphorylation subcellular localization of recombinant wild type S6K1 and mutant form which modulates phosporylated state of the kinase, were compared in the NIH3T3 cells. The localization of kinase was determined by confocal scanning microscopy using appropriate antibodies. As shown in Fig.2 both S6K1 wild type and S6K1S17E localized preferably in the cytoplasm at the absence of growth factors and only in 7% of the cells localized in the nucleus at the same conditions. However, after stimulation with 10% serum for 1 hour the S6K1 wild type translocates to the nucleus in the majority of cells (60%). Appreciable changes in localization of S6K1S17E were not detected.

The data presented show that Ser17 phosphorylation either blocks nuclear import induced by serum or activates nuclear export of S6K1. Regarding the existence of S6K1/CK2 complex only in the nucleus of the cell the latter assumption seems to be more probable. To confirm this assumption the localization of both forms of S6K1 under nuclear export blocking conditions was analyzed.

The protein export from the nucleus is an active process which takes place in several known ways, among which the CRMI-dependant export is the most studied one. We used direct inhibitor of CRMI – LMB [7]. Fig.2

shows that LMB treatment of the cells leads to accumulation of wild type S6K1 in the nucleus both in starvation and stimulation conditions. As it was predicted in the case of S6K1S17E, the significant increase of cells with nuclear localization of the kinase (up to 55%) was observed, which indicates the existence of active import of S6K1S17E mutant to the nucleus. The obtained results allow to make a conclusion that CK2 phosphorylation of S6K1 Ser17 is involved in the regulation of S6K1 export from the nucleus.

To summarize the mentioned-above results we propose the model of regulation of nuclear-cytoplasmic CRM1-dependent S6K1 transport induced by growth factors. The model predicts active, induced by growth factors, import of S6K1 to the nucleus, however the character of impulses and molecular mechanisms of this process are not defined yet. We can suppose that post-translational modifications of S6K1 can fulfill this role. On the other hand, S6K1 export from the nucleus is CRM1-dependent, where the phosphorylation of S6K1 Ser17 by CK2 is a requirement. Taking into account literature data, CK2 activity is constitutive and is not dependent on growth factors, so the regulation of S6K1 export from the nucleus, most likely, takes place on the level of functional CK2/S6K1complex formation.

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Регуляция субклеточной локализации киназы рибосомного белка S6 казеинкиназой 2

Резюме

Казеин киназа 2 (CK2) как физиологически связывающийся партнер киназы S6 рибосомного белка (S6K1) идентифицирована с использованием дрожжевой двугибридной системы. Специфичность взаимодействия между регуляторной b-субъединицей CK2 и S6K1 подтверждена in vitro, кроме того, показано, что S6K1 может быть фосфорилирована CK2 по Ser17. Представленные данные свидетельствуют о том, что фосфорилирование Ser17 – важный этап регуляции экспорта S6K1 из ядра. Исследованиями по иммунопреципитации S6K1 и CK2 из ядерной и цитоплазматической фракций клеток линии NIH3T3 показано существование комплекса S6K1/CK2 в ядре, но не в цитоплазме. С использованием флуоресцентной микроскопии виявлено, что мутант S6K1 (S17E) не накапливается в ядре благодаря активированному экспорту киназы из ядра.

Ключевые слова: S6K1, S6K2, CK2b, ядерный экспорт

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