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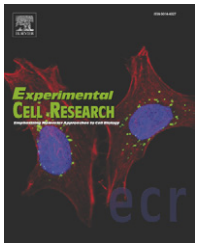

Research Article

Role of eIF3a in regulating cell cycle progression

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ABSTRACT

Translational control is an essential process in regulation of gene expression, which occurs at the initiation step performed by a number of translation initiation factor complexes. eIF3a (eIF3 p170) is the largest subunit of the eIF3 complex. eIF3a has been suggested to play roles in regulating translation of a subset of mRNAs and in regulating cell cycle progression and cell proliferation. In this study, we examined the expression profile of eIF3a in cell cycle and its role in cell cycle progression. We found that eIF3a expression oscillated with cell cycle and peaked in S phase. Reducing eIF3a expression also reduced cell proliferation rate by elongating cell cycle but did not change the cell cycle distribution. However, eIF3a appears to play an important role in cellular responses to external cell cycle modulators likely by affecting synthesis of target proteins of these modulators.

Introduction

Translational control of mRNAs is one of the major regulations of gene expression and it occurs mainly at the translation initiation, the speed-limiting step of protein synthesis. In eukaryotes, there are at least 12 translation initiation factor complexes (eIFs) involved in this complicated procedure [1]. One of these factors, eIF3, is the most complex one with a molecular weight of about 550–700 kDa, consisting of 13 putative subunits known as eIF3a through eIF3m [1,2].

eIF3a (also called eIF3 p170) is the largest and thought to be the major subunit of eIF3 complex initially purified from rabbit reticulocyte lysate [3]. However, its functional importance in translational control and role in the eIF3 complex still remain to be determined. The finding that eIF3a interacts with other subunits of eIF3 [4,5], eIF4B [6], and RNA [5,7] supports a role of eIF3a in the function of the eIF3 complex and in translation initiation. It has also been observed that eIF3 preparations relatively rich in eIF3a did not differ substantially in specific activity of stimulating formation of pre-initiation complexes from preparations that essentially lacked this protein [8], suggesting that eIF3a may have other functions in regulating mRNA translation by binding to other components of the eIF3 complex. Indeed, we recently found that eIF3a regulates the translation of tyrosinated α-tubulin, ribonucleotide reductase M2, and p27 [9,10]. Interestingly, the effect of eIF3a on the translation of p27, tyrosinated α-tubulin, and ribonucleotide reductase M2 are different. Thus, it is possible that the eIF3 complexes with and without eIF3a subunit may be responsible for the translation of different subsets of mRNAs.

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The expression of eIF3a is significantly elevated in several human cancers including breast [11], cervix [12], esophagus [13], stomach [14], and colon (Dong et al., unpublished observations), suggesting that eIF3a may be needed for the malignant growth of tumors. Indeed, knocking down the expression of eIF3a in both breast and lung cancer cell lines reversed the malignant phenotype of these cells [9]. Furthermore, eIF3a has been suggested to be essential for G1-S phase transition in yeast and it may be involved in growth control of yeast cells [15].

Together, these previous findings suggest that eIF3a may be involved in the regulation of cell growth, proliferation and cell cycle progression. In the present study, we investigated the role of mammalian eIF3a in cell cycle control and found that its expression oscillates with cell cycle and peaks in S phase. Down regulating eIF3a expression increased the doubling time without altering cell cycle distribution but changed the sensitivity of cells to environmental stresses that affect cell cycle.

Materials and methods

Materials

Antibodies against actin and GAPDH were from Sigma (ST. Louis, MO) and Abcam (Cambridge, MA), respectively. [^{3}H]thymidine, Hoechst 33342, and the enhanced chemiluminescence (ECL) system for Western blot analysis were from ICN, Invitrogene (Carlsbad, CA) and Amersham Biosciences (Piscataway, NJ), respectively. Sequi-Blot polyvinylidene membrane and concentrated protein assay dye reagents were from Bio-Rad (Hercules, CA). All other reagents were of molecular biology grade and obtained from Sigma or Fisher Scientific (Chicago, IL).

Cell lines, culture, treatment and cell growth rate

NIH3T3 cells were cultured in DMEM containing 10% calf serum and maintained in a humidified atmosphere with 10% CO_2 at 37 °C. H1299 was maintained in RPMI 1640 supplemented with 10% fetal bovine serum in a humidified incubator at 37 °C with 5% CO_2. The H1299 cells stably-transfected with antisense cDNA of eIF3a or vector control, HAS4, HAS5 and HVec, were cultured in RPMI1640 plus 10% fetal bovine serum and 100 mg/ml Zeocin.

Starvation, synchronization and treatment were performed by plating 6×10^5 cells in 10-cm dishes. The cells were allowed to grow for three days in complete medium and the medium was then replaced with that containing 0.1% fetal bovine serum for starvation and synchronization or with fresh complete medium containing mimosine, hydroxyurea, or nocodazole for treatment. Cell growth rate was performed exactly as previously described [9].

Real-Time RT-PCR

Real-Time RT-PCR was performed as described previously [16]. Briefly, total RNAs were extracted using RNeasy Mini Kit (Qiagen). 1 μg of the total RNAs was reverse transcribed to synthesize cDNA using iScriptTM cDNA Synthesis Kit (Bio-Rad). The PCRs were carried out in ABI Prism@7000 Sequence Detection System (Applied Biosystems) using SYBR Green diction according to the manufacturer's instructions. The primers used for eIF3a are 5′-AGATGAGGACAGAGGACCTAGAC-3′ (forward) and 5′-TCAGCATTCCGCCAGGATGA-3′ (reverse) and the primers used for GAPDH control are 5′-AAGGACTCATGACCACAGTCCAT-3′ (forward) and 5′-CCATCACGCCACAGTTTTC-3′ (reverse). The threshold cycle (Ct) was defined as the PCR cycle number at which the reporter fluorescence crosses the threshold reflecting a statistically significant point above the calculated baseline. The Ct of eIF3a was determined and normalized against that of GAPDH internal control. The relative RNA level $=2^{\Delta Ct}$.

Cell cycle analysis, metabolic labeling and sorting

Cell cycle analysis was performed as previously described [10]. Briefly, cells were harvested and washed twice with phosphate-buffered saline (PBS) followed by fixation in 80% ethanol for 30 min at room temperature. The cells were then collected by centrifugation and stained with 50 μg/μl propidium iodide. The cells were then treated with 100 μg/μl RNase for 15 min at 37 °C followed by analysis using a FACScan flow cytometer. Cell cycle distribution was analyzed with the Modfit LT program.

[^{3}H]thymidine labeling was performed by growing 1×10^5 cells/well in a 24-well plate in triplicates for 24 h followed by serum starvation/synchronization for 48 h as described above. The synchronized cells were then released into cell cycle progression by replacing with fresh complete medium and were pulse-labeled for 1 h with 3 mCi/ml [3H]thymidine (60 Ci/mmol) at different time points. The cells were then harvested, counted, and precipitated with 10% TCA. The acid-insoluble material was collected on a filter by rapid filtration and the radioactivity was determined by scintillation counting.

Cell sorting was performed as previously described [17]. Briefly, 5×10^7 cells were washed twice with Hst buffer consisting of Hanks Balanced Salt Solution, 20 mM HEPES (pH 7.2), 1 g/l glucose and 10% fetal bovine serum. After washing, the cells were stained in Hst buffer containing 1.67 μM Hst at 37 °C for 1 h. The cells were then washed twice and resuspended in 2 ml Hst buffer. Cell sorting was performed on a Becton-Dickinson FACSstar^{plus} equipped with a 6 W argon-ion laser emitting 50 mW of ultraviolet (UV 351–356 nm) light. Cells were sorted and collected into three fractions of G0/G1, S and G2/M phase based on the Hoechst 33342 staining intensity. Each fraction was then re-tested to confirm cell cycle and used for preparation of cell lysate and western blot analysis.

Sample preparation and western blot analyses

Sample preparation and western blot analyses were performed as described previously [10]. Briefly, cell lysates were prepared by lysis of cells with TNN-SDS buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.5% Nonidet P-40, 50 mM NaF, 1 mM sodium orthovanadate, 1 mM DTT, 0.1% SDS and 2 mM phenylmethylsulfonyl fluoride) at 4 °C for 30 min followed by centrifugation (10,000 *g* for 10 min at 4 °C) for clearance and protein concentration measurement using Bradford method [18]. The cell lysates were separated by 8% SDS-PAGE and transferred to a PVDF membrane. The blot was then probed with affinity-purified polyclonal antibody AbD (1:1000 dilution) and actin or GAPDH-specific monoclonal antibodies (1:3000 dilution).