FEBS Letters 581 (2007) 5158–5164

Pioneer round of translation mediated by nuclear cap-binding proteins CBP80/20 occurs during prolonged hypoxia

Nara Oh, Kyoung Mi Kim, Junho Choe, Yoon Ki Kim*

School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea

Received 30 August 2007; revised 28 September 2007; accepted 2 October 2007

Available online 11 October 2007

Edited by Lev Kisselev

Abstract Nonsense-mediated mRNA decay (NMD) is one of the mRNA surveillance mechanisms, which eliminates aberrant mRNAs harboring premature termination codons. NMD targets only mRNAs bound by the nuclear cap-binding protein complex CBP80/20 which directs the pioneer round of translation. Here we demonstrate that NMD occurs efficiently during prolonged hypoxia in which steady-state translation is drastically inhibited. Accordingly, CBP80 remains in the nucleus, and processing bodies are unaffected with regard to their abundance and number under prolonged hypoxic conditions. These results indicate that mRNAs enter the pioneer round of translation during prolonged hypoxia.

Keywords: Pioneer round of translation; Nonsense-mediated mRNA decay; Nuclear cap-binding protein CBP80/20; Eukaryotic translation initiation factor; Hypoxia

1. Introduction

The gene expression pathway in mammalian cells involves a number of highly coordinated events. These include transcription, splicing, mRNA export from the nucleus to the cytoplasm, and translation. In addition, mammalian cells have evolved sophisticated mRNA quality control mechanisms that ensure the fidelity of gene expression by recognizing and degrading faulty mRNAs [1]. One of the best characterized mRNA quality control mechanisms thus far is the nonsense-mediated mRNA decay (NMD), which rids the cells of mRNAs that harbor premature termination codons (PTCs) [2,3]. By so doing, NMD eliminates the production of the truncated form of the protein, which could be deleterious to cells. Within the cells, PTCs can arise in transcripts as a consequence of somatic mutations, errors in transcription, inaccurate or inefficient splicing, failure to incorporate selenocysteine at specific UGA codons of some selenoprotein mRNAs, or alternative splicing [2,3].

NMD is preceded by the pioneer round of translation which occurs on newly synthesized mRNAs bound by the heterodimer of the nuclear cap-binding proteins 80/20 (CBP80/20) to the 5′-end cap structure. During the pioneer round of translation, translating ribosomes along the mRNAs displace exon junction complexes (EJCs) deposited onto mRNA as a consequence of the splicing event. Following the completion of the pioneer round of translation initiation, CBP80/20 is replaced by the major cytoplasmic cap-binding protein eIF4E, which directs steady-state translation initiation. Thus, the pioneer round of translation differs from steady-state translation in the following ways [4–6]. First, the pioneer round of translation occurs on newly spliced, EJC- and CBP80/20-bound mRNAs, whereas steady-state translation occurs on EJC-free and eIF4E-bound mRNAs. Second, the pioneer round of translation occurs during mRNA export through the nuclear pore complex, whereas steady-state translation occurs in the cytoplasm. Third, steady-state translation allows for a rapid response to alterations in physiological conditions and environmental stresses [7–10]. With regard to specifically the pioneer round of translation, however, few studies regarding its control under stressful conditions have been conducted thus far.

In this study, we have attempted to determine whether the pioneer round of translation and its consequent event NMD are controlled by cellular stresses, and also have attempted to ascertain the fate of mRNAs synthesized under such stress conditions. In order to answer these questions, we have assessed the efficiency of NMD, which targets the mRNA bound by CBP80/20 but not eIF4E, under prolonged hypoxic conditions in which steady-state translation is blocked to a significant degree [7,8,10,11]. We show that both nucleus-associated and cytoplasmic NMD occur efficiently under prolonged hypoxic conditions. These results indicate that NMD and its upstream event, the pioneer round of translation, occur efficiently under prolonged hypoxic conditions, so as to recognize and degrade aberrant mRNAs. In support of this notion, we also demonstrate that a component of the major nuclear cap-binding protein, CBP80, is highly localized to the nucleus, and that the size and numbers of processing bodies remain unaltered under prolonged hypoxic conditions. On the other hand, eIF4E is redistributed into the nucleus under prolonged hypoxic conditions.

2. Materials and methods

2.1. Cell culture, transfections, and protein and RNA purification

The cultivation of HeLa cells and plasmid transfection were performed as previously described [12]. One day after transfection, cells were grown either under normoxic conditions (20.9% O_2, 5.0% CO_2) or placed in a hypoxic chamber (0.1% O_2, 5.0% CO_2) for 12 h. Total proteins and RNAs were prepared as previously reported [13].

*Corresponding author.
E-mail address: yk-kim@korea.ac.kr (Y.K. Kim).

0014-5793/$32.00
doi:10.1016/j.febslet.2007.10.002

2.2. RNA analysis and luciferase activity assays

Endogenous SMG7, FLuc, β-Gl, GPx1, and MUP mRNAs were amplified as described previously [4,6,12]. In brief, semi-quantitative RT-PCR was performed using specific primer sets and α-[^{32}P]-dATP. As a control for the variation of RNA extraction and sample loading, endogenous SMG7 mRNA was amplified simultaneously. Labeled PCR products were electrophoresed in 5% polyacrylamide gel, visualized by PhosphorImaging (BAS-2500; Fuji Photo Film Co.), and then quantitated by Multigauge (Fuji Photo Film Co.). A standard curve of intensity versus RNA amount was prepared using 2-fold serial dilutions of purified RNAs, and then the relative amounts of PCR products were determined from the curve.

Dual luciferase assays were performed as previously described [4,6,12].

2.3. Western blotting

Western blotting was conducted as described previously [13]. The antibodies used in this study recognized Upf1, Upf2, Upf3X (gifts from Dr. Lynne E. Maquat), CBP80 [14], eIF4AIII [15], HIF-1α (a gift from Dr. Sung-Gil Chi), eIF4E (Cell Signaling), eIF4GI [16], eIF4AI (Abcam), eIF3 (a gift from Dr. John W. Hershey), eIF2α (Cell Signaling), Phospho-eIF2α (Ser51) (Cell Signaling), eIF4E-BP1 (This antibody recognizes both phosphorylated and dephosphorylated form of eIF4E-BP1; Cell Signaling), Phospho-eIF4E-BP1 (Ser65/Thr70) (Santa Cruz), and β-actin (Sigma).

2.4. Immunofluorescence

Immunostaining were performed as previously described [12]. The antibodies used in this study recognized FLAG tag (Sigma), eIF4E (BD Bioscience), and eIF4GI. The primary antibodies were detected using rhodamine- or fluorescein-conjugated secondary antibodies (Pierce). The nuclei were stained with DAPI (Biotium Inc.). Cells were observed with a ZEISS confocal microscope (LSM510 META). When indicated, HeLa cells were transiently transfected with 2 μg of pCDNA3-FLAG-Dcp1a [17] or pCDNA3-FLAG-CBP80 [12] using Lipofectamine 2000 (Invitrogen).

3. Results

3.1. Steady-state translation, but not the pioneer round of translation, is drastically inhibited during prolonged hypoxia

In order to characterize the effects of prolonged hypoxia on the pioneer round of translation and steady-state translation,

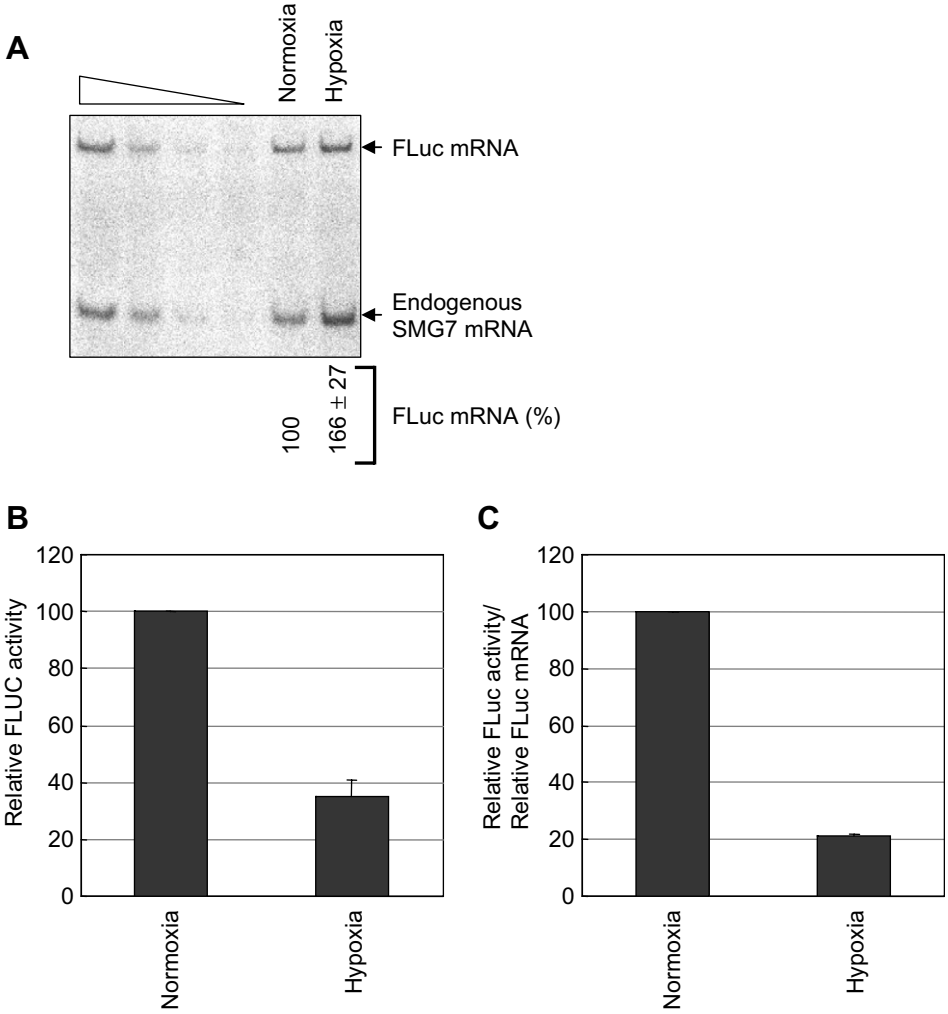

Fig. 1. Prolonged hypoxia reduces the efficiency of steady-state translation. HeLa cells were transiently transfected with a combination of plasmids (see text for the details). One day after transfection, cells were either grown under normoxic conditions or placed into a hypoxic chamber for 12 h. (A) Semi-quantitative RT-PCR of FLuc and endogenous SMG7 mRNAs. The four leftmost lanes represent 2-fold serial dilutions of purified RNAs and demonstrate that the RT-PCR is semi-quantitative. The numbers below the panel represent the level of FLuc mRNA after normalization to the level of endogenous SMG7 mRNA. (B) Relative FLuc activities. FLuc activity was normalized to the level of total protein. (C) The relative FLuc activity was normalized to the level of the relative FLuc mRNA.