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Depletion of cellular poly (A) binding protein prevents protein synthesis and leads to apoptosis in HeLa cells

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ABSTRACT

The cytoplasmic poly (A) binding protein (PABP) is important in mRNA translation and stability. In yeast, depletion of PABP leads to translation arrest. Similarly, the PABP gene in *Drosophila* is important for proper development. It is however uncertain, whether mammalian PABP is essential for mRNA translation. Here we showed the effect of PABP depletion on mRNA metabolism in HeLa cells by using a small interfering RNA. Our results suggest that depletion of PABP prevents protein synthesis and consequently leads to cell death through apoptosis. Interestingly, no detectable effect of PABP depletion on transcription, transport and stability of mRNA was observed.

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1. Introduction

The cytoplasmic poly (A) binding protein (PABP) interacts with eIF4G and the poly (A) tract of mRNA simultaneously to bring its 5′ and 3′ ends into close proximity by forming a closed-loop structure, and stimulates mRNA translation [1] Interactions with additional regulatory proteins allow PABP to regulate the translation initiation step. For example, the Paip1 interacts with PABP and acts like a translational enhancer. Another regulatory protein Paip2 disrupts the interaction of PABP with Paip1, and suppresses PABP-dependent mRNA translation [2]. Furthermore, PABP is believed to protect mRNAs from degradation by binding to their poly (A) tract [3]. Studies have shown that the interaction between PABP and the poly (A) tract may be regulated by PABP interacting partners. For example, the AU rich element binding polypeptide AUF1 may control mRNA stability by binding to PABP [4].

Studies in yeast have shown that PABP is essential for cell survival and its depletion leads to arrest of translation initiation and results in the lengthening of the poly (A) tract [5]. In addition, inactivation of PABP1 gene in *Drosophila melanogaster* by P element insertion was shown to be embryonic lethal [6]. However, a more recent report of PABP depletion in mammalian cells in culture suggests that it may not be essential for mRNA translation [7]. It appears that reduced PABP level causes rapid degradation of Paip2, an inhibitor of PABP function, through the E3 ubiquitin ligase pathway, and thus allows the remaining PABP to work more efficiently. However, these results are paradoxical because for only approximately ten percent of the remaining PABP to maintain the same rate of mRNA translation, its activity should have to increase by almost ten folds. There is no evidence to support that such a profound increase of PABP activity could occur in the absence of sufficient Paip2. Therefore, we report here a re-examination of the effect of PABP depletion on mRNA metabolism in a mammalian cell culture model. We show that although PABP depletion decreases Paip2 level, this was not sufficient to support mRNA translation, and consequently leads to cell death. Surprisingly PABP depletion had no effect on the transcription, cytoplasmic transport and stability of mRNA transcripts.

2. Materials and methods

2.1. Plasmids and siRNA

A tetracycline-regulated mono and dicistrionic expression vector namely, pTR5-DC/GFPQ*TK/hygro and pTR5-DC/hsp7-GFP/hygro derived from plasmid pTR-DC/GFP were used in this study [8]. The siRNA sequence for silencing PABP expression and the control siRNA were the same as described by Yoshida et al., 2006 [7] and purchased from Dharmacon (Thermo Fisher Scientific, Dharmacon product, Crescent Drive, Lafayette, CO).

2.2. Cell culture and siRNA transfection

HeLa Cells were grown in Dulbecco's modified Eagle's medium (DMEM) including 1% glutamine (Sigma, Oakville, Canada) and 10% fetal bovine serum (VWR, Mississauga, ON, Canada) in a

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humidified 5% CO_2 incubator at 37 °C. Subsequently, cells were transfected at 30–50% confluence using Lipofectamine 2000™ (Invitrogen, Burlington, ON, Canada) according to manufacturer's (Invitrogen) instructions. For each transfection, 1.5 μl of siRNA duplex (20 μM annealed duplex from Dharmacon) was used with 5 μl of Lipofectamine 2000™ Co-transfection of the cells was performed as described above, only with the exception of adding 1 μg of either plasmid pTR5-DC/GFPQ*TK/hygro or pTR5-DC/hsp7-GFP/hygro DNA in the transfection complex. Seventy two hours after transfection, the medium was changed with fresh DMEM medium incubated with doxycycline (1 μg/ml, Sigma) for an additional 24 h.

2.3. Western blotting

Cells were lysed with 200 μl of 1 × SDS gel-loading buffer (50 mM Tris–HCl, pH 6.8; 2% (w/v) sodium dodecyl sulfate (SDS); 0.1% (w/v) bromophenol blue; 10% (v/v) glycerol and 5% (v/v) 2-mercaptoethanol). The samples were boiled for 5 min and separated by SDS-10% polyacrylamide gel electrophoresis (PAGE), and transferred to nitrocellulose membrane. The desired protein was detected with a specific primary antibody, followed by incubation with an appropriate HRP-conjugated secondary antibody. The membrane was developed with western lighting chemiluminescence reagent plus (PerKinElmer LAS, Inc., Shelton, USA) and quantified by ImageJ program.

2.4. Immunofluorescence and Confocal microscopy

Cells were grown on glass cover slips placed in 35 mm tissue culture dishes and transfected as described above. Cells were fixed with 4% Paraformaldehyde and used for either immunostaining or *in situ* hybridization for as previously described [9]. The localization of proteins was visualized and imaged with a Leica Microsystems Confocal Laser Scanning Microscope (CLSM, Microsystems, Inc., Heidelberg, Germany) equipped with a Plan-Achromat 63x/NA1.4 objective. Optical sections of representative cells were obtained by laser confocal fluorescence microscopy (CLFM) as previously described [9]. Two hundred cells in different fields of view were visualized and representative images of the majority of cells were used for presentation. Controls were performed by using non-immunized serum. In order to avoid cross-talks between FITC and Texas red channels, the signals were scanned individually only with only one channel on and the other off. The images for the same cells from two channels were merged with overlap function. To differentiate the auto-fluorescence, control cells without immunostaining were examined to set up the settings for the stained cells.

2.5. In situ hybridization

A molecular beacon oligodeoxynucleotide probe was used to detect the cytoplasmic localization of the β-actin mRNA. The 5′ end of the probe was labeled with a fluorochrome and the 3′ end with the Dabcyl quencher. The nucleotide sequence of the probe

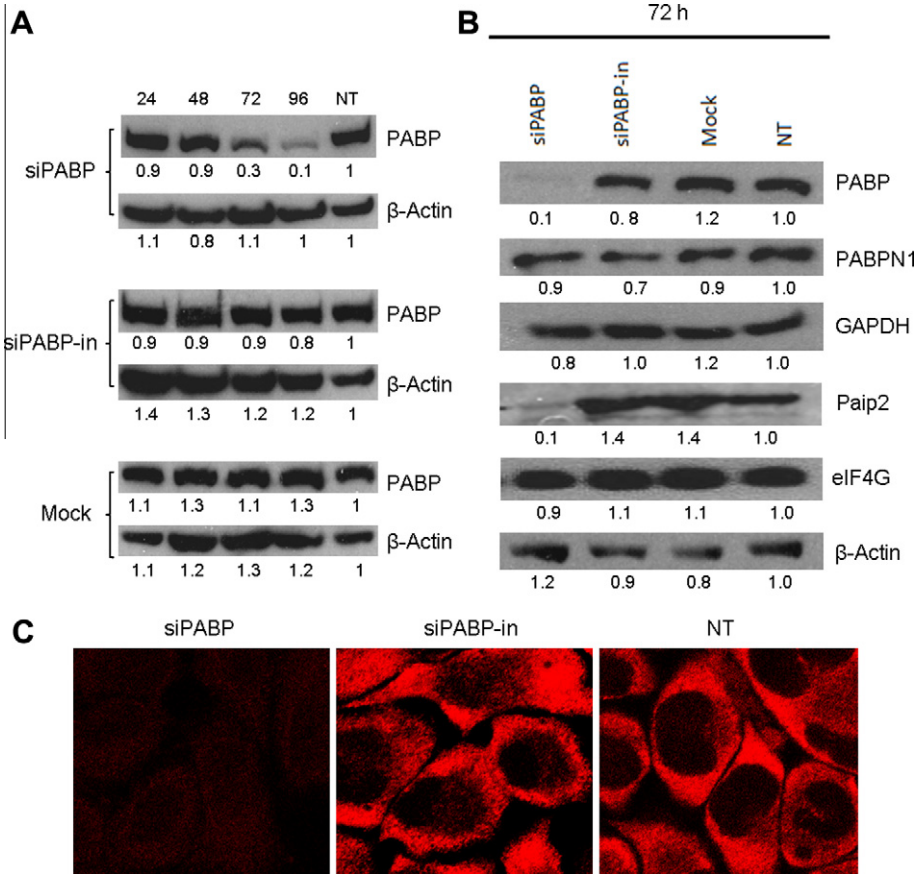

Fig. 1. PABP knock down with siRNA. (A) Hela cells were transfected with siPABP, and the abundance of various polypeptides was examined by western blotting as described in experimental procedures using appropriate antibodies. Samples from cells transfected with the anti PABP siRNA (siPABP), control siRNA (siPABP-inv), and non transfected (NT) cells were examined. (B) Samples from siRNA transfected ells following 72 h of treatment and control cells were examined for the abundance of PABP, PABPN1, GAPDH, Paip2, eIF4G, and β-actin by western blotting. (C) siRNA transfected cells were immunostained with PABP antibody, followed by incubation with a Texas red conjugated secondary antibody. Images were taken by a laser scanning confocal microscope as described in experimental procedures. The results (panels A–C) are representative of three independent experiments.

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