Investigating the role of Ribonuclease Inhibitor 1 in TOP-sequence mediated translation and its interactions with poly(A)- binding protein

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1. Abstract

Ribonuclease Inhibitor 1 (RNH1) has been identified as playing a critical role in the translation process in higher vertebrates. It has been demonstrated that RNH1 deficiency leads to a reduction in global translation rates specifically in the erythroid lineage, while other tissues remain unaffected by this translational defect. This impairment is likely due to disrupted mRNA circularization, which results from decreased interactions between key translation initiation factors, such as the cytoplasmic poly(A)-binding protein (PABPC) and eukaryotic initiation factor (eIF) 4G. Previous research has established that RNH1 and PABPC interact. The aim of this study was to determine which domain of PABPC binds to RNH1 using overexpression of both proteins followed by co-immunoprecipitation (co-IP). The experimental results suggest that the interaction between RNH1 and PABPC is likely a secondary interaction mediated by another protein, rather than a direct binding event.

2. Introduction

RNH1 is a cytoplasmic protein that inhibits the activity of ribonucleases and plays a crucial role in the process of translation [1]. RNH1 deficiency leads to a reduction in the global translation rate specifically in erythropoietic cells, while other tissues remain unaffected by this translational defect. Co-IP experiments using endogenous cell lysates from hematopoietic cell lines, such as K562, demonstrated that RNH1 interacts with several translation initiation factors, including PABPC, eIF4G, and La-related protein 1 (LARP1) [2]. These interactions contribute to mRNA circularization by bringing the 3' end into proximity with the 5' end through a series of interactions, involving the 3'-poly(A) tail, PABPC, eIF4G, eIF4E, and the 5' cap. These interactions are essential for translation initiation and the maintenance of translation efficiency in eukaryotic cells [3]. Interestingly, in RNH1 knockout (KO) K562 cells, the interaction between PABPC and eIF4G was reduced. This decrease in PABPC-eIF4G interaction may have impaired mRNA circularization, leading to reduced translation in RNH1 KO hematopoietic cells [2].

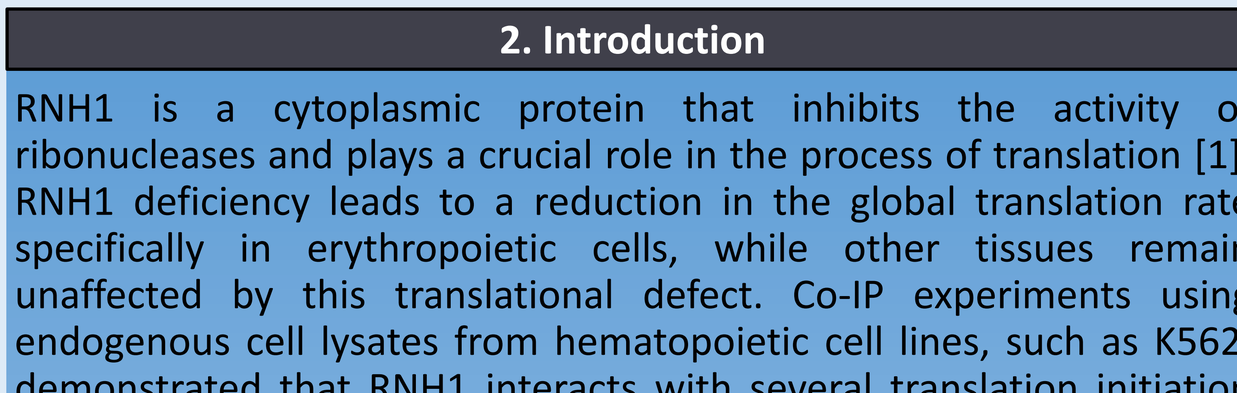


Figure 1: mRNA circularization in different RNH1 scenarios. **A)** RNH1 wild-typ (WT) **B)** RNH1 KO (Stillinovic et al., 2024, adapted and created with BioRender.com).

PABPC is critical for both translation and mRNA decay and is composed of four tandemly repeated N-terminal RNA recognition motifs (RRMs), which are essential for binding the 3' poly(A) tail of most mRNAs. Following the RRMs is an unstructured linker region that contains a C-terminal PABC domain. The linker region is involved in the formation of PABPC multimers, while the PABC domain facilitates various protein-protein interactions [4].

3. Aim & leading Question

This thesis endeavors to make a modest contribution to the elucidation of the protein RNH1, specifically delving into interactions with PABPC.

- Which domain of PABPC binds to RNH1 to promote mRNA circularization?

4. Methods & Material

To evaluate which domain of PABPC binds to RNH1, WT HEK293T cells were co-transfected with two plasmids: one for overexpressing RNH1 and the other for either the full-length PABPC protein, the four N-terminal RRM domains, or the C-terminal linker region containing the PABC domain. The required plasmids, which include a flag tag for subsequent identification of the overexpressed proteins, were synthesized using classical restriction cloning and the Gateway cloning technique. The RNH1 overexpression plasmid was previously synthesized by other lab members. 24 hours post-transfection, cell lysates were prepared and subjected to co-immunoprecipitation targeting the flag tag. Finally, the samples were analyzed by Western blotting to detect protein binding.

5. Results

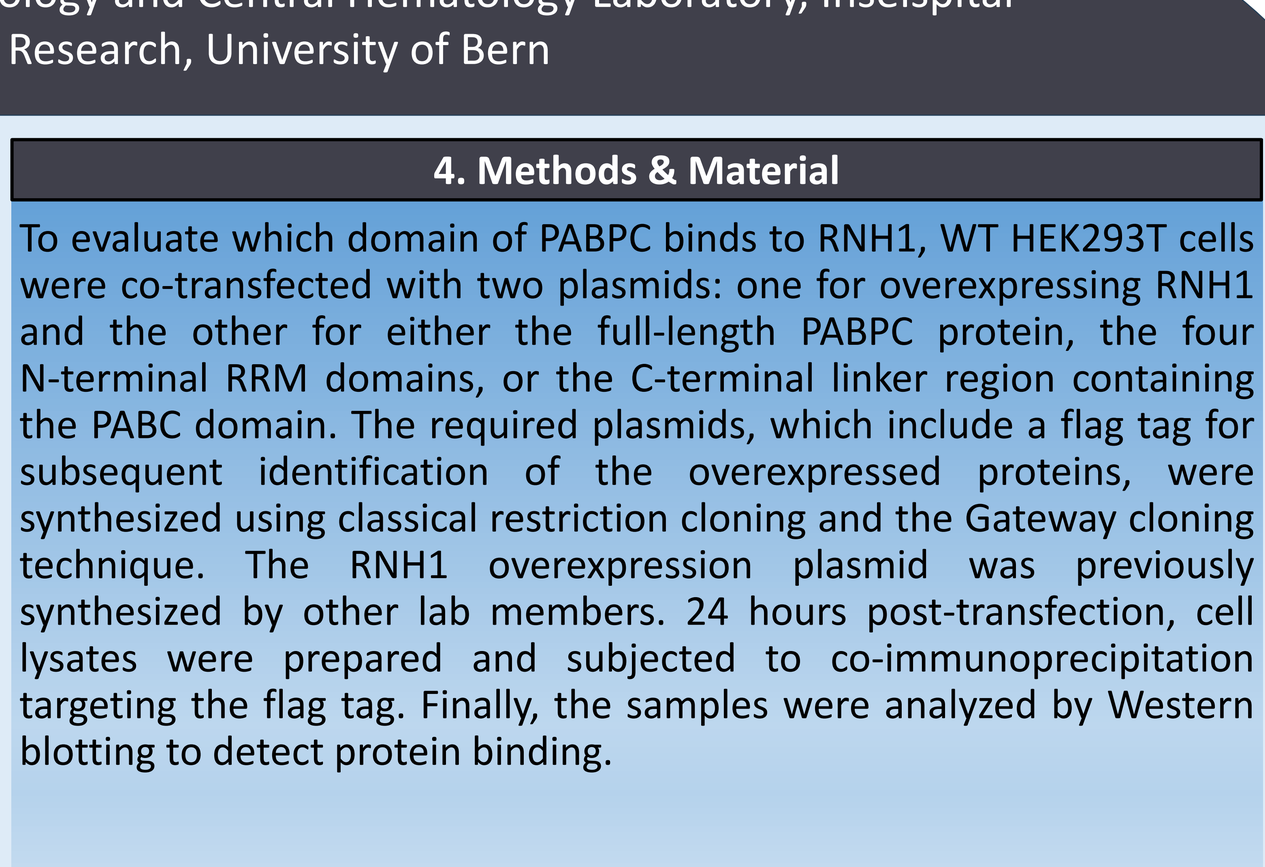


Figure 2: Western blot analysis of FLAG co-IP with the lysate of HEK293T cells 24 after transfection (Zatti, 2024).

6. Discussion & Conclusion

The co-IP assay for flag tag revealed no detectable binding between RNH1 and PABPC, including both the full-length protein and its individual domains, as indicated by the absence of 50 kDa bands. Notably, the lack of binding for the full-length protein contrasts with previous studies. These experiments exclusively involved endogenous PABPC and RNH1, whereas the current study utilized overexpression systems, which may disturb the natural cellular equilibrium. Furthermore, the artificial flag tagging of proteins may alter their interaction dynamics. Given the involvement of these proteins in translation, it is plausible that their interactions are mediated by secondary binding to mRNA or other proteins. Previous studies have shown diminished interactions between PABPC and eIF4G or LARP1 in RNH1 knockout cells, suggesting that, under physiological conditions, interaction may occur between RNH1, PABPC, eIF4G, and LARP1. In the current experimental setup, however, there is a lack of LARP1 or eIF4G. LARP1, in particular, is a compelling candidate for secondary interactions due to its PABPC-interacting motif, a hypothesis that warrants future experimental validation.

List of references:

[1] Haigis et al., 2002; https://doi.org/10.1093/oxfordjournals.molbev.a004153
[2] Stillinovic et al., 2024; https://doi.org/10.1126/sciadv.adl0320
[3] Cockman et al., 2020; https://doi.org/10.3390/biom10070969
[4] Kajjo et al., 2022; https://doi.org/10.15252/embj.2021108650

List of figures:

Figure 1: Stillinovic et al., 2024; https://doi.org/10.1126/sciadv.adl0320
Figure 2: Zatti, M. (2024). Western blot examination of HEK293T cell lysates 24 hours post-transfection. medi.