Evaluation of Eukaryotic Translation Initiation Factor 5A (EIF5A) in the translation of S6Ks proteins

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Introduction: The eukaryotic translation initiation factor 5A (EIF5A) is highly conserved in eukaryotes, which has a particular amino acid residue named hypusine not found in others proteins. Recent studies have shown that EIF5A plays a critical role in translation elongation of proteins containing consecutive proline residues. The protein S6K2 has a region with five consecutives prolines in its C-terminus. The S6Ks are responsible for phosphorylating the ribosomal protein S6, and this activation is essential for protein synthesis, and cell cycle progression. In addition, it has been suggested that proline-rich regions can be part of a regulatory mechanism for protein activity. However, there are no reports demonstrating this mechanism for S6K2. Objectives: The aim of this project is to evaluate the importance of poli-proline stretch for S6K2 synthesis and activity, seeking a greater understanding of its regulation. Material and Methods: A mutant S6K2 (S6K2ΔPro) was produced changing the poly-proline region to the corresponding sequence present in the homologous S6K1. Assays were performed to evaluate the production and ability of the mutant S6K2ΔPro to phosphorylate S6, as compared to S6K1 and S6K2. HeLa cells were suppressed of eIF5A, by esiRNA treatment, and the endogenous production of S6K2 evaluated. Results and Discussion: The S6K2ΔPro was produced similarly to S6K2 and it was able to phosphorylate the protein S6. In addition, knockdown assays showed that the suppression of eIF5A did not reduce the endogenous production of S6K2 under the conditions tested. Conclusions: It was observed that the production of the S6K2 protein is not dependent of eIF5A and even without the poly-proline sequence, S6K2 is still capable to phosphorylate S6 protein. Additional trials are being conducted using a model of yeast knockdown for eIF5A homologous and immunoprecipitation assays in order to compare the protein interactome of S6K2 and S6K2ΔPro.

Key words: protein synthesis, poly-proline, EIF5A, S6K.
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