ALANINE SCANNING MUTAGENESIS TO STUDY eIF5A BINDING TO THE RIBOSOME BY FLUORESCENCE ANISOTROPY

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The translation factor 5A (eIF5A) is highly conserved in Archaea and eukaryotes and is essential for cell viability. This is the only protein known to contain the amino acid residue hypusine, generated by a post-translational modification and necessary for eIF5A maturation. Although eIF5A has been involved in several cellular processes, it was only recently determined a role for eIF5A in protein synthesis, more specifically, in the elongation cycles. It was suggested that, during translation elongation, eIF5A enhances the peptide bond formation of specific amino acid sequences. To improve the description and understanding of the mechanism of eIF5A in translation, it is necessary to determine the points of interaction in both ribosome and eIF5A. Our laboratory and collaborators have previously developed a fluorescence anisotropy assay to measure the kinetics of eIF5A binding to the ribosome. We chose one representative alanine scanning mutant of each different growth phenotype to evaluate their ribosome binding ability in vitro: Cluster 2, temperature sensitive; Cluster 3, dominant negative; Cluster 5, no growth phenotype; and Cluster 7, slow growth. Using ultracentrifugation on a sucrose cushion, it was shown the reduced ability of mutants Cluster 2 and 7 to bind to 80S. Subsequently, the binding affinity was measured by competition assays by fluorescence anisotropy. All four mutants tested in this work showed reduced affinity for the ribosome, however, the mutant Cluster 7 showed the lowest interaction with the ribosome. Thus, we were able to demonstrate that mutations in determined charged residues of eIF5A affect binding to the ribosome and those residues in the mutant Cluster 7 are the most important for eIF5A binding to 80S.

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