

STRUCTURAL MODELING AND MUTATIONAL ANALYSIS OF YEAST EIF5A REVEAL NEW ESSENTIAL RESIDUES

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eIF5A is a conserved protein essential for cell viability, but its biological role of is unclear. This factor is the only protein that undergoes hypusination, a process involving the enzymes Dys1 and Lia1. In this work, the tertiary structure of yeast eIF5A was modeled based on the structure of its *Leishmania mexicana* homologue. This model was used to predict the localization of mutations generated randomly by EMS or PCR-based mutagenesis and to design new site-directed eIF5A mutants. We obtained and characterized 43 mutants and their growth phenotypes were analysed: 19 are totally non-functional; 14 mutants are temperature-sensitive; and 10 showed no obvious phenotype when compared to wild type. It was also assayed their ability to interact physically with Dys1 and at which extent they serve as substrate for hypusination. Also, we determined the protein stability of eIF5A mutants and identified two eIF5A mutants with stable protein at the non-permissive temperature, which will be important for further eIF5A functional analysis. In summary, these new described eIF5A mutants were very useful in structural protein analysis and may contribute to better understand eIF5A function.

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