Phenotypic Characterization of Deoxihypusine Synthase Conditional Mutants

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Eukaryotic translation initiaton factor (eIF5A) is an essential and highly conserved protein present in all organisms from archaea to mammals. Although eIF5A has already been involved with different functions in the cell, recent studies favor a role for eIF5A in translation. This factor is the only protein known to contain the unique and essential amino acid residue hypusine, formed by a posttranslational modification reaction that involves two enzymatic steps: initially deoxyhypusine synthase (Dys1) transfers the aminobutyl moiety from the polyamine spermidine to the amino group of one specific eIF5A lysine residue (K51) to form deoxyhypusine. In the second step, deoxihypusine hydroxylase (Lia1) completes hypusine synthesis (eIF5A maturation). Although the mechanism of deoxyhypusine synthesis (hypusination) has been extensively characterized, the role of the hypusine residue is still obscure. To better understand the function of hypusine, in this work we generated two temperature-sensitive mutants of *DYS1*: *dys1*$^{?1\text{-}28}$ and *dys1-1* (*dys1*$^{W75R/T118A/A147T}$), a conditional mutant that grows only in the presence of an osmotic stabilizer. The *dys1-1* shows a dramatic decrease in Dys1 protein levels, which may result in very low levels of hypusinated eIF5A, while the *dys1*$^{?1\text{-}28}$ mutant demonstrates increased binding to eIF5A and may sequester functional eIF5A in the cell. These mutants are being used in several functional assays and phenotypic characterization to contribute to the understanding not only of the hypusine function, but also of the eIF5A role in the cell.

Keywords: eIF5A, Hypusination, *DYS1*, *Saccharomyces cerevisiae*

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