

# **NMR based Metabolic Profiling of *Saccharomyces cerevisiae* with High Levels of mRNA Mistranslation.**

Claudio H. Santos<sup>1</sup>, Brian J. Goodfellow<sup>1</sup>, João Paredes<sup>2</sup> and Manuel A.S. Santos<sup>2</sup>.

<sup>1</sup>CICECO, Dept. de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

<sup>2</sup>CESAM, Dept. de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal

High mRNA translation fidelity is critical for the production of stable and functional proteomes and, therefore, mRNA mistranslation events that produce aberrant proteins are often linked to disease. In normal physiological conditions mRNA mistranslation errors are low ( $10^{-4}$  to  $10^{-5}$ ), however, the error frequency increases under stress, aging and certain diseases. In order to understand this phenotype at the molecular level, we have induced constitutive mRNA mistranslation in *Saccharomyces cerevisiae*, using tRNA engineering methodologies (1).

By using NMR spectroscopy at high field, the effect of mRNA mistranslation on the *Saccharomyces cerevisiae* metabolome was studied. Using a method similar to that used in Lewis et al (2), low MWt (< 5kDa), metabolites were obtained and a series of 1D spectra and, on selected samples, 1H-13C-HSQC spectra were acquired. Using a PCA approach, the separation of samples from control cells and samples from cells with different levels of mRNA mistranslation was achieved. The loadings from the PCA plots indicated that separation was due to differing levels of trehalose, glycerol, acetic acid and a number of amino acids. These results are consistent with previous studies that showed important differences in gene expression in mistranslating cells (1). Comparison of these data from mistranslating cells with data from other types of stress, namely heat shock, will allow better characterization of the *Saccharomyces cerevisiae* stress response at the metabolome level.

In parallel, complete identification of the low MWt metabolites, visible by NMR, is being carried out using 1D/2D NMR in conjunction with LC-NMR methods. A previous study identified ca 40 metabolites. This has now been expanded to ca 50 using coupled NMR methods.

[1] Silva RM, et al. EMBO J (2007) vol. 26 (21) pp. 4555-65

[2] Lewis IA et al. Anal Chem (2007) vol. 79 (24) pp. 9385-90