

P011 The application of LC-MS-MS to study translation initiation complexes and their altered modification status in glucose-replete and -deplete yeast cells

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Several cellular stresses have been shown to induce a global inhibition of protein synthesis at the level of translation initiation, allowing the rapid adaptation to environmental change. The mechanisms involved in the inhibition of protein synthesis have been characterized for some of these stresses, e.g. amino acid starvation in yeast. The depletion of glucose results in both the most dramatic inhibition of translation observed in yeast to date and a subsequent transcriptional derepression of ~30% of yeast genes which function in alternative carbohydrate metabolic pathways. However, the mechanism involved in the translational inhibition remains unknown and forms the basis of this work. Several key translation initiation factors have been TAP tagged and purified. Purified complexes have been digested using trypsin for direct analysis with LC-MS-MS to assess the purified complexes and their modification status. MASCOT datasets have been acquired for these translation initiation factor complexes. This analysis is currently being extended to incorporate isotopic protein labeling which will additionally allow for a quantitative analysis of both the tagged and interacting translation initiation factors in glucose replete and deplete cells. Hence we aim to compile a comprehensive list of alterations both in the abundance and modification status of translation initiation factors following glucose starvation in yeast.