QUANTITATIVE ANALYSIS OF DIFFERENTIAL PHOSPHORYLATION IN A PKC1 OVEREXPRESSION STRAIN OF SACCHAROMYCES CEREVISIAE

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In S. cerevisiae, protein kinase C (Pkc1p) is involved in the control of actin polarization and morphogenesis. Pkc1p acts upstream of the cell integrity MAPK pathway. A protein kinase C overexpression strain of S. cerevisiae was investigated for differential protein phosphorylation as compared to an isogenic wild type strain.

We have used a phosphoproteomic approach based on quantitative mass spectrometry based on stable isotope labeling with amino acids in cell culture (SILAC).

The PKC1 overexpression strain was labeled by growth in media containing stable isotopic amino acids, i.e C13 – arginine and C13-lysine, to do differential analysis in a 1:1 protein mixture of both strains using mass spectrometry.

Several phosphopeptide enrichment techniques have been used, and all fractions were analysed by nano – HPLC-MS/MS and neutral loss dependent MS3 on a LTQ mass spectrometer that allowed identification of phosphopeptides using Mascot scoring and quantification with MSquant, a freely distributed program for SILAC quantification.

Of 299 non-redundant phosphopeptides identified and quantified, 93 were upregulated more than 2-fold (average ratio).

The proteomic work was done at the Proteomics Facility of UCM-PCM, a member of ProteoRed Network.