

## STUDY OF PROTEIN KINASE C SIGNALLING IN SACCHAROMYCES CEREVISIAE BY SILAC-BASED PHOSPHOPROTEOMICS

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*Saccharomyces cerevisiae* has been widely used as a model eukaryotic organism to elucidate the molecular mechanisms involved in signal transduction pathways mediated by mitogen-activated protein kinase (MAPK) cascades. Five MAPK pathways have been described in this organism: namely the pheromone, filamentous and invasive growth, spore wall assembly, high osmolarity glycerol (HOG) and cell wall integrity (CWI) pathways. Protein kinase C (Pkc1) is essential to activate the MAPK module in the CWI pathway but it is assumed to regulate yet unknown additional targets distinct from the MAPK cascade downstream. The CWI pathway, mediated by the Slt2 MAPK, has been studied by our group in depth for a long time. Although much knowledge exists on the function and regulation of this pathway, direct phosphorylation targets of both Pkc1 and Slt2 are still largely ignored.

As an approach to understand phosphorylation changes induced by activation of these kinases we have performed a comparative phosphoproteomic study, based on quantitative mass spectrometry using stable isotope labeling by amino acids in cell culture (SILAC), which has revealed differential phosphorylation in peptides corresponding to Pkc1 and Slt2 themselves, as well as proteins related to morphogenesis and actin cytoskeletal function, signalling, translational and transcriptional control, ubiquitination, protein folding and metabolism. Further directed small-scale analyses *in vivo* by immunoblotting have verified particular phosphorylation events on account of Pkc1-dependent signalling, results which prompt us to study their functional implications.