

IPD Project Details

Project ID: IPD1627

Project Title: Identification of phosphorylated amino acids in CgSub2 protein in *Candida glabrata*

Description: The objective of the project is to identify the phosphorylated amino acid residues in CgSub2 in the pathogenic yeast *Candida glabrata*. For this analysis, wild-type cells and mutants lacking CgHog1 MAPK or CgSlt2MAPK or both CgHog1 and CgSlt2 MAPKs were used.

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Sample Preparation: For phosphorylation site identification in CgSub2, the overnight cultures of wt, Cghog1?, Cgslt2? and Cghog1?slt2? strains expressing C-terminally SFB (S protein-Flag-Streptavidin-binding peptide)-tagged CgSub2 (CgSub2-SFB) were grown in fresh CAA medium for 5 h at 30°C. Cells were harvested and lysed in NETN buffer [Tris-HCl (20 mM; pH 8.0), NaCl (100 mM), EDTA (1 mM) and Nonidet P-40 (0.5%)] using glass beads. Whole cell lysates were subjected to tandem affinity purification with streptavidin-agarose and S-protein-agarose beads. Briefly, 15 mg protein per lysate sample was first incubated with streptavidin beads for 2 h at 4°C, followed by elution in the biotin (2 mg/ml)-containing buffer. The resulting supernatant was incubated with S-protein agarose beads for 2 h at 4°C. After washes, beads were boiled, and proteins were resolved on a 10% SDS-PAGE gel. The gel was stained with Coomassie Brilliant Blue and destained.

Peptide Separation: The 65 kDa band, corresponding to CgSub2-SFB, was excised from the gel, placed in water and sent to the Taplin Biological Mass Spectrometry (MS) facility at Harvard Medical School, Boston, USA for identification of phosphorylated residues in CgSub2 protein via LC-MS/MS analysis. Sample information is detailed below: wt/CgSUB2-SFB (Raw name-79565 and Search name-56911), Cghog1?/CgSUB2-SFB (Raw name-79566 and Search name-56914), Cgslt2?/CgSUB2-SFB (Raw name-79567 and Search name-56913) and Cghog1?slt2?/CgSUB2-SFB (Raw name-79568 and Search name-56912).

Protein Characterization: All generated fragmentation patterns were acquired and searched against the UniProt C. glabrata reference proteome database using the SEQUEST software to determine the peptide sequences. A modification of 79.9663 mass unit to serine, threonine and tyrosine was considered for phosphorylation. Ascore algorithm was used for phosphorylation assignments. The data was filtered to 1-2% peptide false discovery rate.

Experiment Type: Bottom-up

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: iodoacetamide derivatized residue

PubMed ID: