

IPD Project Details

Project ID: IPD3501

Project Title: CgSub2 interactome analysis in the presence and absence of CgHog1 MAPK in *Candida glabrata*

Description: The objective of the project is to identify CgSub2-interacting proteins in the presence and absence of CgHog1 MAPK in the pathogenic yeast *Candida glabrata*, with CgSub2 being an interactor of CgHog1.

Principal Investigator: Dr Rupinder Kaur

PI Affiliation: Laboratory of Fungal Pathogenesis, Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad, India

Sample Preparation: For CgSub2 interactome analysis, the overnight cultures of wt and Cghog1[?] strains expressing C-terminally SFB (S protein-Flag-Streptavidin-binding peptide)-tagged CgSub2 (CgSub2-SFB) or C-terminally SFB-tagged GFP (GFP-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, 8 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 resultant 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 till bromophenol blue dye in the sample buffer entered about 3 mm into the gel. The gel was stained with Coomassie Brilliant Blue and destained. The portion of the gel, that displayed protein bands, was excised, placed in water and sent to the Taplin Biological Mass Spectrometry (MS) facility at Harvard Medical School, Boston, USA for protein identification via LC-MS/MS analysis. Samples were prepared and sent for analysis from two independent biological replicates.

Peptide Separation: At the Taplin facility, gel pieces were subjected to in-gel trypsin digestion followed by microcapillary LC-MS/MS (Liquid chromatography-tandem mass spectrometry) using the LTQ Orbitrap Velos Pro ion-trap mass spectrometer. Sample information is detailed below: wt/CgSUB2-SFB_Replicate-1 (Raw name-79569 and Search name-56801), Cghog1[?]/CgSUB2-SFB_Replicate-1 (Raw name-79570 and Search name-56808), wt/GFP-SFB_Replicate-1 (Raw name-79571 and Search

name-56807), Cghog1?/GFP-SFB_Replicate-1 (Raw name-79572 and Search name-56806), wt/CgSUB2-SFB_Replicate-2 (Raw name-79573 and Search name-56805), Cghog1?/CgSUB2-SFB_Replicate-2 (Raw name-79574 and Search name-56804), wt/GFP-SFB_Replicate-2 (Raw name-79575 and Search name-56803), Cghog1?/GFP-SFB_Replicate-2 (Raw name-79576 and Search name-56802).

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. The data was filtered to 1-2% peptide false discovery rate.

Experiment Type: Bottom-up

Species: Data in species_details No Data

Tissue: Unknown No Data

Cell Type: Unknown No Data

Disease: Unknown No Data

Instrument Details: LTQ Orbitrap Velos (MS:1001742) Data in instrument_details

Protein Modifications: dehydrated residue

PubMed ID: